

U.S. Biodiesel Development: New Markets for Conventional and Genetically Modified Agricultural Products. James Duffield and Hosein Shapouri, Office of Energy, Economic Research Service, U.S. Department of Agriculture; Michael Graboski and Robert McCormick, Department of Chemical Engineering and Petroleum Refining, Colorado School of Mines; and Richard Wilson, Agricultural Research Service, U.S. Department of Agriculture at North Carolina State University. Agricultural Economic Report No. 770.

Abstract

With environmental and energy source concerns on the rise, using agricultural fats and oils as fuel in diesel engines has captured increasing attention. Substituting petroleum diesel with biodiesel may reduce air emissions, increase the domestic supply of fuel, and create new markets for farmers. U.S. agricultural fats and oils could support a large amount of biodiesel, but high production costs and competing uses for biodiesel feedstocks will likely prevent mass adoption of biodiesel fuel. Higher-priced niche markets could develop for biodiesels as a result of environmental regulations. Biodiesel has many environmental advantages relative to petroleum diesel, such as lower CO, CO₂, SO_x, and particulate matter emissions. Enhancing fuel properties by genetically modifying oil crops could improve NO_x emissions, cold flow, and oxidative stability, which have been identified as potential problems for biodiesel. Research activities need to be directed toward cost reduction, improving fuel properties, and analyzing the economic effects of biodiesel development on U.S. agriculture.

Keywords: Biodiesel, biodiesel blends, fatty acid esters, soybean, oil crops, animal fats, plant genetics, diesel engines, alternative fuel

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Summary

The development of diesel fuels made from fats and oils derived from crop and animal products could create new markets for U.S. farmers and help reduce harmful emissions from diesel engines, but high production costs and competing uses for feedstocks will likely prevent mass adoption of such “biodiesel” fuels.

The annual U.S. supply of fats and oils averages about 3.7 billion gallons. If used for biodiesel feedstock, this would amount to about 13 percent of the 28 billion gallons of diesel fuel used in the United States each year. In 1998, the U.S. biodiesel industry had an annual capacity of about 60 million gallons. Biodiesel and biodiesel blends could mitigate some of the environmental effects of diesel fuel use. Biodiesel producers have to compete with other buyers of fats and oils in agricultural commodity markets. Oil crops and animal fats are produced mainly for domestic livestock feed (meal from oilseed crops and grain from corn), food products, industrial purposes, and for export.

Prices of vegetable oils and animal fats are greater than the market price of diesel fuel. Soybean oil commands \$1.50 per gallon more than No. 2 diesel; lard, tallow, and yellow grease cost less than vegetable oils, but they are still priced above diesel fuel. The total cost of converting soybean oil to biodiesel would be about \$2.52 per gallon. A less expensive feedstock such as yellow grease would cost about \$1.39 per gallon of biodiesel, still about 82 cents higher than the wholesale price of petroleum diesel.

Potential biodiesel feedstocks produced in the United States include soybeans, canola, peanut, corn, and cottonseed and animal fats such as tallow, yellow grease, and lard. The two major oil crops, soybeans and corn, are grown mostly in the north-central region of the Nation. Texas, California, and Mississippi are the largest cottonseed producers. The biggest peanut States are Georgia, Texas, and Alabama, while most sunflower seeds are grown in the Dakotas. Tallow and lard are products of slaughter facilities, which are concentrated in the midsection of the United States. Yellow grease, primarily recycled cooking grease, can be found nationwide.

Higher priced niche markets could develop for biodiesel and biodiesel blends as a result of the Clean Air Act Amendments of 1990 and the energy-security provisions of the Energy Policy Act of 1992. Some consumers might voluntarily pay premium prices for biodiesel’s demonstrated environmental benefits—improved biodegradability, reduced carbon monoxide and sulfur oxide emissions, reduced odor, reduced particulate emissions, less soot, and safer handling than petroleum diesel.

Biodiesel’s performance versus petroleum (No. 2) diesel as a fuel in diesel engines varies with the feedstocks used:

- Biodiesel (especially those made from highly saturated feedstocks) may cause cold-weather engine problems;
- Biodiesels’ engine ignition delay is generally shorter than No. 2 diesel;

- Biodiesels often emit higher levels of nitrous oxide, a regulated emission;
- Biodiesels are flammable at higher temperatures than No. 2 diesel;
- Fuel oxidation, which happens more quickly with biodiesels, decreases storage life; and
- Biodiesel offers improved engine lubrication to reduce engine wear.

Genetic modification of the fatty acid composition of oilseeds has become standard research, especially as intended for edible products. Biotechnology can also modify soybean and other vegetable oil plants to become better feedstocks for biodiesel. Plant breeding and molecular genetics may alter the fatty acid composition of methyl esters to reduce some of the problems associated with biodiesel. Genetic engineering of feedstocks may improve biodiesel's fuel properties to a level equal to or even surpassing petroleum diesel fuels. For example, soybean germplasm with gene combinations can enable a higher degree of saturation to improve oxidative stability, ignition quality, and NO_x emissions. In addition, manipulating other genes can improve the cold-flow properties of biodiesel.

Biodiesel producers are concentrating their marketing efforts on blends, such as a 20-percent biodiesel with petroleum, because they are much cheaper than pure biodiesel and can still significantly reduce some air emissions relative to petroleum diesel. In the past few years, biodiesel and biodiesel blends have been sold mainly for demonstration and testing purposes. A substantial market has not yet emerged. Research activities need to be directed toward cost reduction, quality control, and decreased biodiesel NO_x emissions. And the effect on the agricultural sector from increasing the demand for agricultural oils as fuel feedstocks should be fully investigated. For example, how much biodiesel would have to be produced to significantly increase soybean prices, raise farm income, and spur rural economic growth?

U.S. Biodiesel Development: New Markets for Conventional and Genetically Modified Agricultural Fats and Oils

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Introduction

Since Rudolf Diesel invented his engine over 100 years ago, the diesel engine has been able to use vegetable oil as fuel. However, as petroleum became the dominant energy source for the world, petroleum diesel was developed as the primary fuel for diesel engines.

Research on vegetable oils as a diesel fuel fluctuated over the years, with interest peaking during emergency situations, such as World Wars I and II and the energy crisis of the 1970's, when petroleum fuel supplies were interrupted. More recently, issues related to the environment and energy security have brought to the fore alternative fuels such as ethanol, natural gas, and biodiesel. Legislation, such as the Clean Air Act Amendments (CAAA) of 1990 and the Energy Policy Act of 1992, has opened markets for alternative fuels that can be produced from domestic resources and that are more environmentally benign than petroleum-based fuels. In addition, many U.S. farmers and policymakers support the development of renewable fuels, such as biodiesel and corn ethanol, as a means of creating new markets for agricultural commodities.

Although diesel engines will run on whole vegetable oil fuels, tests conducted in the 1930's indicated engine problems occur with long-term use of these fuels (Walton). More recent tests show that prolonged use of these types of fuels results in incomplete combustion,

causing severe engine deposits, ring sticking, injector coking, and eventually engine failure (Peterson et al., 1996). These problems can be alleviated by modifying the oil through a process called transesterification, which reacts an agricultural oil with an alcohol (e.g., ethanol or methanol) in the presence of a catalyst to produce glycerol and a fatty alkyl ester. These alkyl esters can be used as a fuel that is commonly referred to as biodiesel. Biodiesel is a substitute for petroleum diesel and can be used in most diesel engines with only minor modifications. Glycerol, a valuable coproduct, is used in pharmaceuticals, cosmetics, toothpaste, paints, and other commercial products.

Biodiesel may be made from many crops grown in the United States, including soybeans, corn, cottonseed, sunflowerseed, and peanuts. Soybean oil accounts for about 75 percent of the Nation's crop oil production; oilseed-crushing facilities produce close to 15 billion pounds annually. Corn is the second largest source of U.S. vegetable oil, with about 2 billion pounds of oil produced each year. All other oil crops yield only about 2.8 billion pounds of oil. However, many of these oil crops have a relatively high oil content per acre and may be ideal for biodiesel production. Animal fats such as tallow, lard, and yellow grease (primarily refined used restaurant grease), all of which are plentiful in the United States, can also be used to make biodiesel.

Due to the large amount of soybean production in the United States, biodiesel research and development has focused on biodiesel made from soybean oil (American Biofuels Association). One notable exception is rapeseed oil—the University of Idaho has been conducting research on biodiesel made from rapeseed since 1979 (Peterson et al., 1996). Biodiesel made from rapeseed oil is also widely used in Europe, where tax incentives have encouraged the use of biodiesel. Little research has been done on biodiesel use of other crop oils, e.g., sunflowerseed oil and peanut oil (USDA, ARS, 1983; Swenson et al.). The high cost of vegetable oils has spurred interest in developing less expensive feedstocks for biodiesel, and recent research has demonstrated that tallow and recycled greases are good sources for biodiesel (Foglia et al.; Nelson et al.; Peterson et al., 1996; Ali et al.).

Most biodiesel produced today is blended with petroleum diesel at a 20-percent level (B20) and is used primarily for testing and demonstration. Biodiesel demand from these activities, along with expectations of increasing demand in the next few years, has encouraged some investment in biodiesel production. A biodiesel industry with an annual capacity of about 60 million gallons has emerged (*Biofuels Update*, 1997). A purpose of this report is to provide a general overview of biodiesel development in the United States and explore the economics of using various agricultural feedstocks for biodiesel production. We look at the availability of agricultural fats and oils as biodiesel feedstocks and examine the current markets for these commodities. In addition, we identify yield differences among agricultural feedstocks and the potential for increasing yields through plant breeding and genetic modifications. Fuel properties and chemical composition are summarized to allow quality comparisons among feedstocks, and the potential for genetically engineering improved feedstocks is explored. Finally, we discuss research proposals aimed at developing new technologies that may advance the commercialization of biodiesel, along with economic analyses to estimate the effect of increased biodiesel demand on existing agricultural markets.

Oil Crops Production

Soybeans and Corn

The two major oil crops in the United States, soybeans and corn, are grown mostly in the north-central region of the Nation (USDA, NASS, 1996). The biggest pro-

ducers of soybeans in the United States are Illinois and Iowa, at about 11 million tons each per year in recent years (fig. 1). Annual soybean production in Indiana, Minnesota, Ohio, and Missouri ranged from 4.2 to 6.4 million tons (1993-95 average). These States—together with Nebraska, Arkansas, and South Dakota—produced about 80 percent of U.S. soybeans.

The next largest source of vegetable oil, corn, is grown throughout the United States but is concentrated in many of the same States as soybeans (fig. 1 and 2). Average corn production in Illinois and Iowa was about 39 million tons and 37 million tons, respectively, between 1993 and 1995. Nebraska produced about 26 million tons, and Minnesota, Indiana, Ohio, and Wisconsin each produced from 9 to 18 million tons of corn annually (fig. 2). These seven States produced about 73 percent of U.S. corn production.

Other Oilseeds

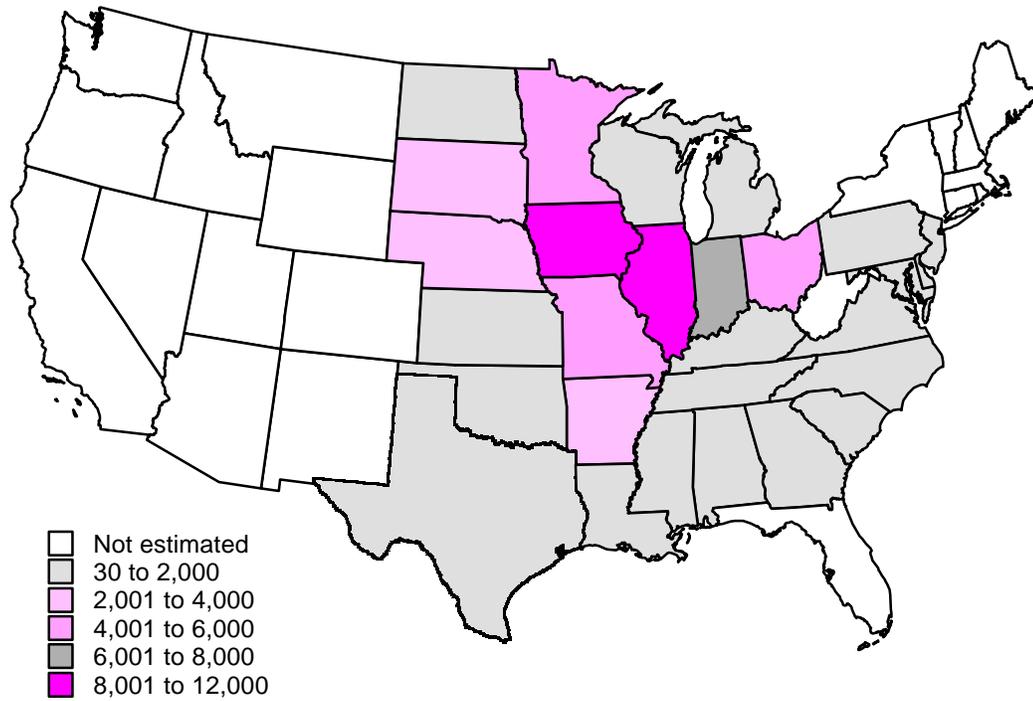
The United States also grows cottonseed, peanuts, and sunflowerseed throughout the country (USDA, NASS, 1996). Just over 10 million tons of oilseed are produced from these crops (fig. 3). Texas, the only State that grows all three of these oil crops, produces mainly cottonseed—Texas grew about 2 million tons of cottonseed annually from 1993 to 1995. Other large producers of cottonseed are California and Mississippi, which annually produced about 1 million and 736,000 tons respectively from 1993 to 1995. Arkansas and Louisiana grew about 500,000 tons annually; Georgia, Arizona, and Tennessee grew between 400,000 and 300,000 tons; North Carolina, Alabama, Missouri, and South Carolina grew between 250,000 and 100,000 tons; and Oklahoma, New Mexico, Florida, Virginia, and Kansas grew less than 100,000 tons.

From 1993 to 1995, the annual average production of U.S. peanuts was about 1.85 million tons. Among the nine States that grow peanuts, Georgia is the largest producer, with almost 800,000 tons of peanuts per year from 1993 to 1995. Georgia's large peanut crop, along with some cottonseed production, makes it the second largest producer, behind Texas, of "other" oilseed crops in the United States (fig. 3). Texas and Alabama are the second and third largest producers of peanuts, growing about 284,808 and 233,800 tons respectively. Other States that grow peanuts include North Carolina, Oklahoma, Virginia, Florida, New Mexico, and South Carolina.

Figure 1

Soybean Production, 1993-95 average (1,000 tons)

Illinois and Iowa each produced about 11 million tons of soybeans per year

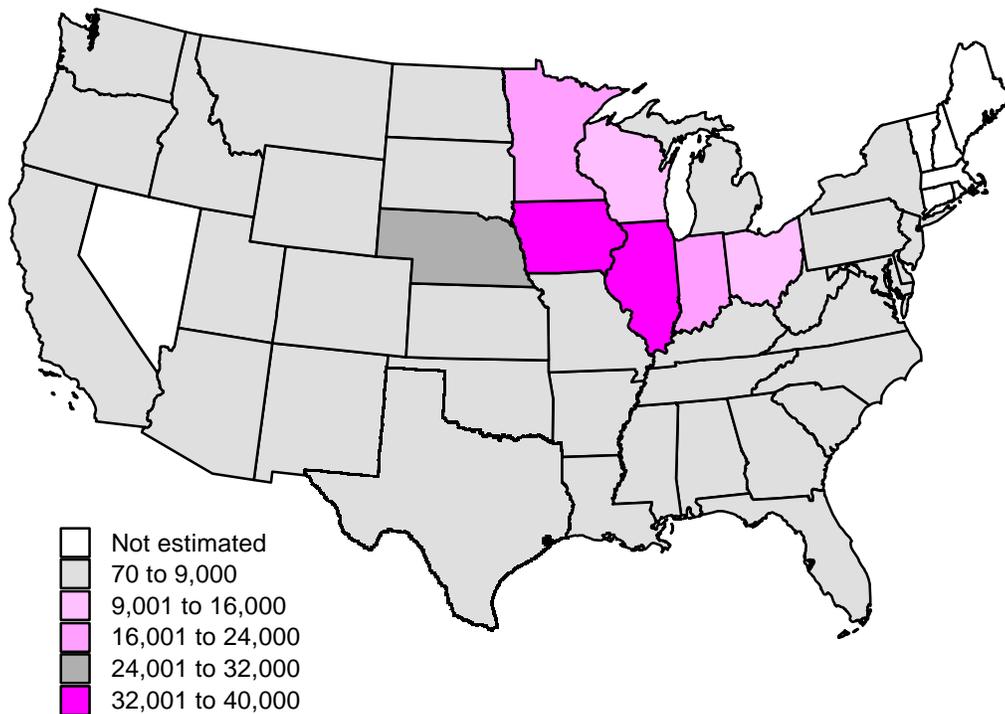


Source: USDA, NASS, 1996.

Figure 2

Corn Production, 1993-95 average (1,000 tons)

Seven States produce about 73 percent of total U.S. corn production

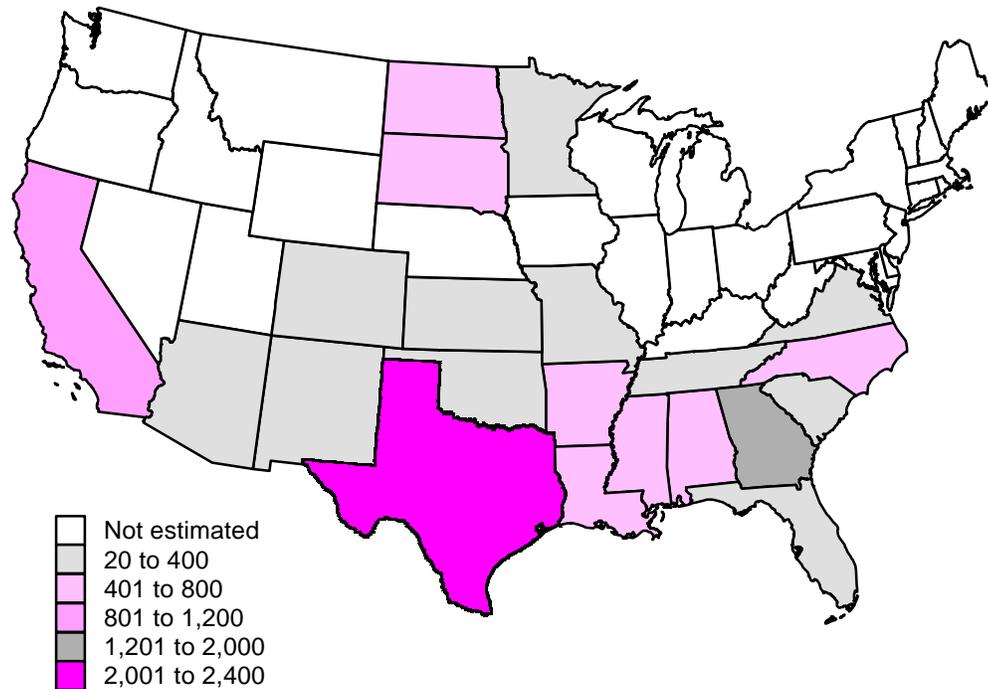


Source: USDA, NASS, 1996.

Figure 3

Other oilseeds production, 1993-95 average (1,000 tons)

Over 10 million tons of oilseed are produced from cottonseed, peanuts, and sunflowerseed each year



Source: USDA, NASS, 1996.

The two largest producers of sunflowers, North Dakota and South Dakota, produced about 704,000 and 548,000 tons of sunflowerseed (for oil use) per year, respectively, about 80 percent of total U.S. annual production from 1993 to 1995. Minnesota, Kansas, Colorado, Nebraska, and Texas also grow sunflowers for oil.

Although production is quite small, U.S. farmers also grow special oilseeds such as safflower, canola, industrial rapeseed, and flaxseed, the source for linseed oil. Since only a few farmers produce these crops, the National Agricultural Statistics Service (NASS) reports only total U.S. production per crop, but not by State, with the exception of flaxseed. However, the 1992 Census of Agriculture recorded State data for some of these crops (fig. 4). NASS recorded a 1993-95 average annual safflower production of 230,000 tons (USDA, NASS, 1996). According to the 1992 Census, safflower was grown in California, North Dakota, Montana, South Dakota, Arizona, Utah, and Idaho. The average production of U.S. canola was 208,000 tons; North Dakota produced the most canola, which was also grown in Washington, Idaho, Montana, Minnesota, Oregon, Georgia, and Alabama. The 1992 Census reported rapeseed production for only Idaho

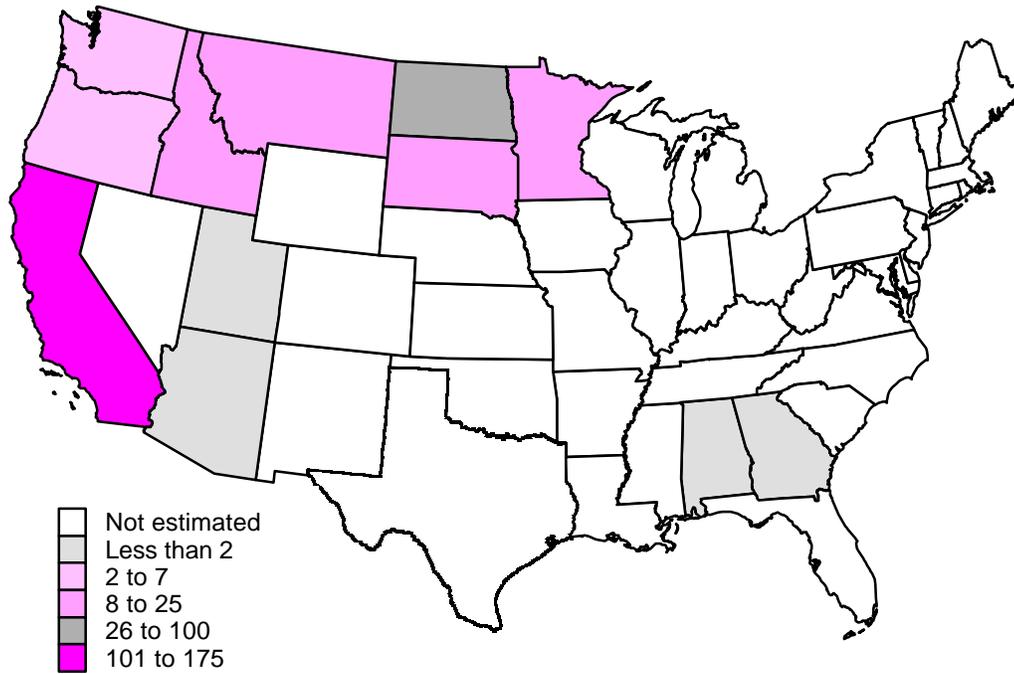
and North Dakota—total U.S. production of rapeseed was about 4,000 tons per year. North Dakota grew about 80 percent of the U.S. flaxseed crop. The only other flaxseed States specifically reported by NASS are South Dakota and Minnesota; however, NASS also reports a small amount of flaxseed grown in “other States.” The annual average amount of flaxseed grown in the United States from 1993 to 1995 was 80,000 tons per year.

U.S. Sources of Tallow, Lard, and Yellow Grease

Most of the 2.56 million tons of tallow produced in the United States each year is obtained from cattle slaughter facilities. About 513,000 tons of lard is produced each year in the United States, coming mostly from hog slaughter facilities. The largest producers of animal fats are Iowa and Nebraska, each producing 18 and 16 percent of the U.S. total, respectively (fig. 5). Kansas and Texas are also large producers, accounting for 14 and 12 percent of the U.S. livestock slaughter, respectively. Colorado, Illinois, and Minnesota each

Figure 4
Special oilseed crop production, 1992 (1,000 tons)

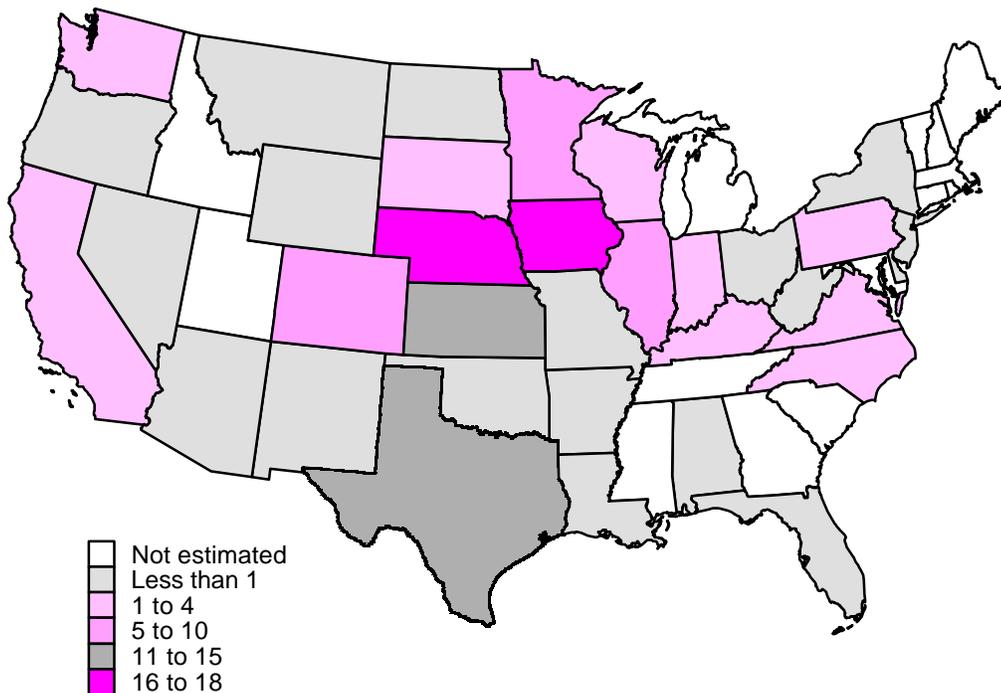
Rapeseed, canola, and flaxseed were grown primarily in the Northern United States; safflower was produced mainly in the north and California



Source: 1992 Census of Agriculture.

Figure 5
Percentage of total U.S. livestock slaughter by weight for each State, 1994

Seven central States produce 75 percent of the Nation's lard and tallow



Source: U.S. Department of Agriculture, NASS, 1995.

produce 4 to 5 percent of the U.S. total. Together, these seven States, located in the midsection of the United States, produce about 75 percent of the Nation's lard and tallow.

Yellow grease is primarily refined cooking grease recycled from restaurants, which place used cooking grease in containers that are emptied into trucks and taken to renderers for processing. Yellow grease can be found throughout the country, with high concentrations of it located in large population areas. The average annual production of yellow grease in the United States from 1993 to 1995 was about 1.3 million tons (U.S. Department of Commerce).

Total U.S. Supply of Biodiesel Feedstocks

Table 1 shows the amount of oil produced annually in the United States by type of feedstock. Amounts are reported by weight (i.e., millions of pounds), which are converted to a liquid volume basis to provide a

Table 1—Supply of potential biodiesel feedstocks

Oil type	Total oil production ¹	
	Pounds	Gallons ²
	Millions	
Crops		
Total	20,030	2,601.3
Soybean	14,935	1,939.6
Corn	2,076	269.6
Cottonseed	1,220	158.4
Sunflowerseed	868	112.7
Canola	353	45.8
Peanut	282	36.6
Flaxseed/linseed	175	22.7
Safflower	118	15.3
Rapeseed	3	0.4
Animal fat		
Total	8,772	1,139.2
Lard	1,026	133.2
Edible tallow	1,490	193.5
Inedible tallow	3,623	470.5
Yellow grease	2,633	341.9
Total supply	28,802	3,740.5

¹Pounds of oil production are a 3-year average (1993-1995) from *Oil Crops Yearbook*, October 1997, USDA, ERS with the following exceptions: rapeseed was calculated by multiplying oil per acre times the 1993-95 average number of acres harvested. Number of harvested acres comes from USDA, NASS, January 1996. Inedible tallow and yellow grease supply comes from U.S. Department of Commerce, Bureau of the Census, *Fats and Oils, Production, Consumption and Stocks, Annual Summaries 1993-1995*.

²Pounds are converted to gallons of oil using a 7.7 pounds-to-gallon conversion rate.

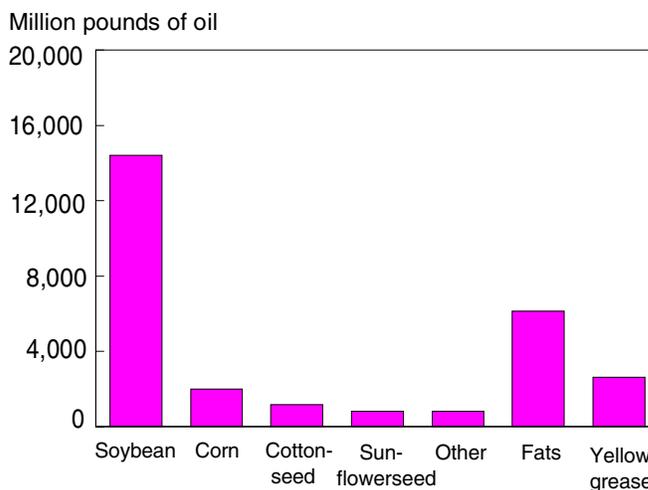
gallon estimate for each feedstock. Soybean oil is the single largest potential feedstock source for biodiesel, with an average annual production of about 14.9 billion pounds from 1993 to 1995 (fig. 6). The 3-year average annual production of corn oil, the next largest source of vegetable oil, was just over 2 billion pounds. Cottonseed and sunflowerseed are also relatively large contributors to U.S. vegetable oil supplies—annual production of cottonseed oil is over 1 billion pounds and sunflowerseed is crushed into 868 million pounds each year. The production of oil from the other feedstocks is minor, ranging from 353 million pounds for canola to around 3 million pounds for industrial rapeseed. The total production of oil from crops is about 20 billion pounds per year. Animal fats and yellow grease add about another 8.8 billion pounds, resulting in about 29 billion pounds of total oil.

On a liquid fuel basis, these feedstocks would equal about 3.7 billion gallons of diesel fuel, about 13 percent of the 28 billion gallons of diesel fuel consumed in the United States for transportation in 1996 (fig. 7). If biodiesel were blended with petroleum diesel fuel, e.g., 20-percent biodiesel and 80-percent petroleum (B20), the total supply of this blended fuel would be about 18.7 billion gallons, or 67 percent of U.S. annual diesel consumption. This example uses the total average supply of all crop oils, animal fats, and yellow grease as the available feedstock supply. Assuming that 10 percent of U.S. feedstocks would be available,

Figure 6

U.S. supply of potential biodiesel feedstocks

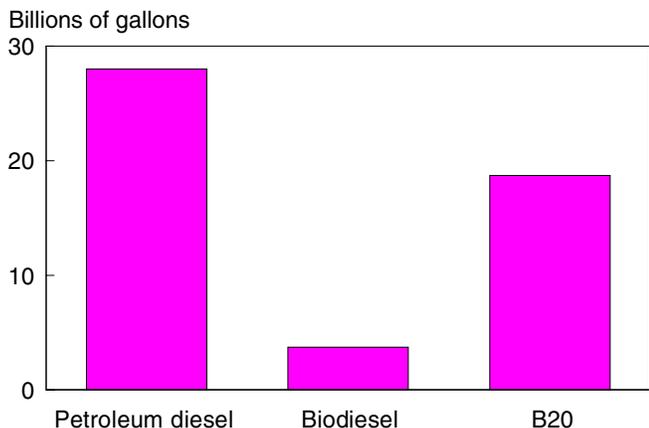
Total production of oil from potential biodiesel feedstock is about 29 billion pounds per year



the supply of neat (100 percent) biodiesel would equal about 1.3 percent of petroleum diesel consumed annually (fig. 8). On a B20 basis, biodiesel would equal about 7 percent of total diesel consumption.

Figure 7
Annual consumption of diesel and potential biodiesel supply using all feedstocks

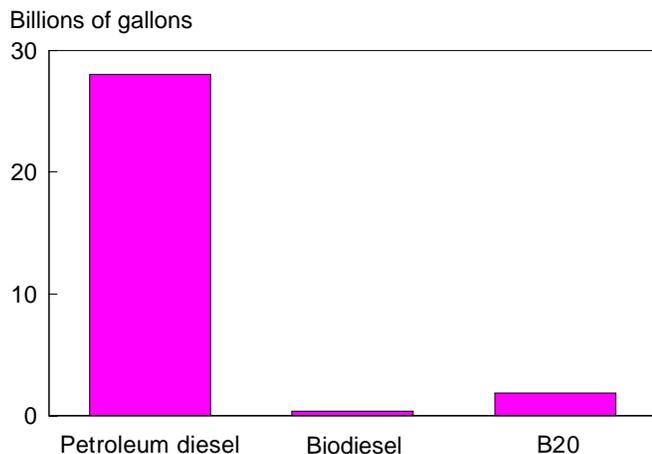
All biodiesel feedstocks combined equal about 13 percent of total U.S. diesel fuel consumption in 1996



Source: U.S. Department of Transportation.

Figure 8
Annual consumption of diesel and potential biodiesel supply using 10 percent of available feedstocks

The supply of B20 would equal about 7 percent of total diesel consumption



Source: U.S. Department of Transportation.

Current Uses of Potential Feedstocks

Biodiesel producers have to compete in established markets for fats and oils with other users of biodiesel feedstocks. While a potential fuel source, oil crops and animal fats are produced mainly for domestic livestock feed, food products, and industrial purposes. Meal from oilseed crops and grain from corn are primarily used for animal feed. The oils from crops and animal fats are used mostly for food products, as well as for industrial uses and animal feed (fig. 9). A large amount of these commodities are exported.

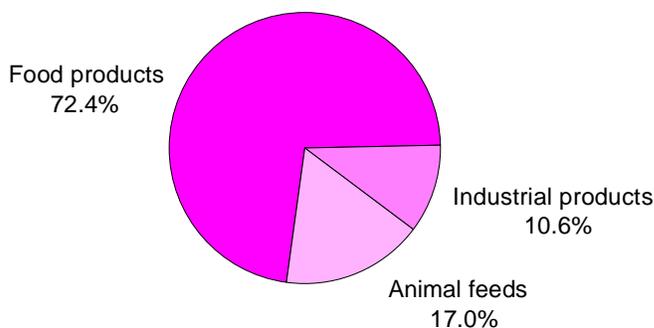
Soybeans

Soybeans are split into three major products: unprocessed, meal, and oil. A third of the soybeans grown on U.S. farms are directly exported without any processing. Most of the remaining soybeans are crushed into oil and meal for domestic use, with a small amount of beans used for seed and whole seed feeding to livestock (fig. 10). About 15 billion pounds of soybean oil is produced annually with about 13 billion pounds consumed domestically and 2 billion pounds exported. About 32 million tons of meal are produced—26 million tons are used domestically and 6 million tons are exported.

Soybean meal is the most valuable product derived from processing the soybean, ranging from 50 to 75 percent of its monetary value. Soybean meal is pri-

Figure 9
Domestic use of fats and oils, 1995

Oils from potential biodiesel feedstocks have edible and inedible uses

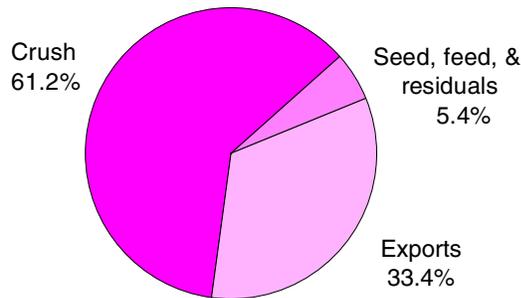


Source: U.S. Department of Agriculture, Economic Research Service, *Oil Crops Yearbook*, October 1997.

Figure 10

Use of U.S. soybeans

More than half of the U.S. soybean crop is crushed into meal or oil



Source: U.S. Department of Agriculture, Economic Research Service, *Oil Crops Yearbook*, October 1997.

marily used as an animal feed and is the world's number one source of protein feed (Ash et al.). The other major product derived from the soybean, soybean oil, has a variety of edible and inedible uses. About 97 percent of U.S. soybean oil is used for edible purposes, making it the largest source of edible oil (about 73 percent). Edible uses of soybean oil include salad and cooking oils, baking and frying fats, and margarine. Inedible uses of soybean oil include a wide variety of industrial applications that use vegetable oil derivatives called oleochemicals; these products include soap, cosmetics, surfactants, lubricants, paints and varnishes, solvents, resins and plastics, stabilizers, emulsifiers, pesticides, and fatty acids. Inks made from soy oil are being used for newspaper printing. However, the industrial markets are not large—only about 300 million pounds of domestically produced soybean oil is used for industrial purposes, which is less than 3 percent of total consumption (Ash et al.).

Corn

Total consumption of U.S. corn is about 8.6 billion bushels per year. Almost 58 percent is used for domestic animal feed, about 23 percent is exported, and 19 percent is used for other domestic purposes. Other domestic uses of corn include starch, high-fructose corn syrup (HFCS), alcohol, glucose, dextrose, cereals, and corn oil. About 2 billion pounds of corn oil is produced annually, of which 60 percent is consumed domestically and 40 percent is exported.

Other Oilseed Crops

Demand for cottonseed and oil-type sunflowerseed includes domestic processing for oil, domestic meal production for animal feed, and unprocessed seed to be crushed abroad. About 22 percent of the 1993-95 annual average cottonseed oil production was exported and 80 percent of sunflowerseed oil production was exported. These vegetable oils are chiefly used in edible products such as salad and cooking oils, margarine, and baking and frying fats. Small amounts are used in industrial products, including plastics, soaps, and animal feeds.

Peanuts are used mostly as an edible nut, both in-shell and shelled, and in other edible products such as peanut butter, peanut candy, and snack peanuts (Sanford and Evans). U.S. peanuts not eligible for the edible channel are crushed into oil and meal or exported, competing in the same food and feed markets as soybeans and other oil crops. Total disappearance for U.S. peanuts was about 4 billion pounds per year from 1993 to 1995. About 2 billion pounds were used for food consumption, about 880 million pounds (22 percent) were crushed into meal and oil, approximately 750 million pounds of peanuts were exported, and 300 million pounds were associated with seed, loss and shrinkage, and residual. Average annual oil production over this time period was 282 million pounds (USDA, ERS, 1997).

Special Oilseed Crops

Linseed oil, obtained from the seed of the flax plant, possesses a drying property that makes it an ideal ingredient in protective coatings such as linoleum and oil-based paints. However, new products have replaced linseed oil as a protective coating. Linseed oil lost the largest market share to latex paints and vinyl floor coverings, which replaced linoleum. Today, most linseed oil is used by the paint industry and some is used for plastics. A small amount is exported.

Safflower is grown mainly for its oil; whole seed safflower is 20 to 35 percent high-quality oil. The oil is used for human consumption, in salad oils, margarine, and similar products, and for industrial use in making paints and soap. The United States is a net importer of safflower oil, importing about 10,000 tons annually from 1993 to 1995.

Rapeseed is used for edible oil and meal; some varieties produce oil suitable only for industrial uses. Rapeseeds contain erucic acid, which may present

health risks to humans and reduce the palatability and nutritional value of livestock feed. While many parts of the world produce high-erucic-acid varieties for human consumption, low-erucic varieties such as canola dominate production for Canada and Western Europe. Because oils high in erucic acid have a high degree of lubricity, they function exceptionally well as direct lubricants or in lubricant formulations (Carlson and Van Dyne). Only about 5,000 acres of rapeseed were harvested in the United States annually from 1993 to 1995. Much of this rapeseed was used in research projects aimed at developing industrial uses for rapeseed oil.

Canola, a rapeseed variety developed by Canadian researchers, is very low in erucic acid. The demand for canola seed is driven mainly by its use in production of canola oil, a popular cooking oil, followed by canola meal. Canola meal is a less valuable livestock feed than soybean meal because it is lower in protein and has a higher roughage content. From 1993-95, annual U.S. canola oil production was 353 million pounds; about 975 million pounds were imported from Canada (USDA, ERS, 1997). Although U.S. canola production is relatively small, it has been the fastest growing oil crop in the United States. Canola production grew from no production in 1986 to 321 million pounds in 1995.

Tallow and Lard

Tallow and lard are used like crop oils. Edible tallow is used for human consumption (e.g., shortening), but some goes to industrial uses and animal feed. About 12 percent of annual U.S. lard production and 20 percent of edible tallow is exported. A large amount (60 percent) of inedible tallow is exported, while the remainder is used domestically for animal feed, soaps, and other industrial uses. Yellow grease, collected from restaurants and other places where used cooking grease accumulates and delivered to renderer plants, is refined and used for making animal feeds, pet food, fatty acids, soap, paints, plastics, lubricants, and other industrial products. About 13 percent of annual U.S. yellow grease production is exported.

Feedstock Prices

Wholesale prices for vegetable oils are driven mostly by the U.S. soybean market (Morgan). Accounting for about one-quarter of world oilseed production, U.S. soybean output helps to determine soybean oil price and influences prices of other vegetable oils. U.S.

vegetable oil prices have fluctuated with no apparent pattern from 1978 to 1995 (fig. 11). The average soybean oil price during this period was about 23 cents per pound, compared with 25 cents per pound for corn, cottonseed, and sunflowerseed. Peanut oil prices move with the other prices but at a higher level—the average price of peanut oil was about 35 cents per pound (fig. 11). Peanut oil commands a higher price because some consumers are willing to pay more for its cooking properties and peanut flavor.

In recent years, oils low in saturated fats have been receiving a price premium over other oils, especially in the United States (Morgan). Health officials have been encouraging Americans to eat less saturated fats and cholesterol. Consequently, vegetable oils low in saturated fat, like canola, safflower, and sunflower oils, generally command higher prices than other crop oils (fig. 12). Canola contains only 6 percent saturated fat, the lowest of all vegetable oils. Safflower oil contains 9 percent saturated fat and sunflower oil contains 11 percent. Soybean, corn, peanut, and cottonseed oils contain saturated fat levels of 15, 13, 18, and 27 percent, respectively.

Relative Prices of Agricultural Feedstocks and Diesel Fuel

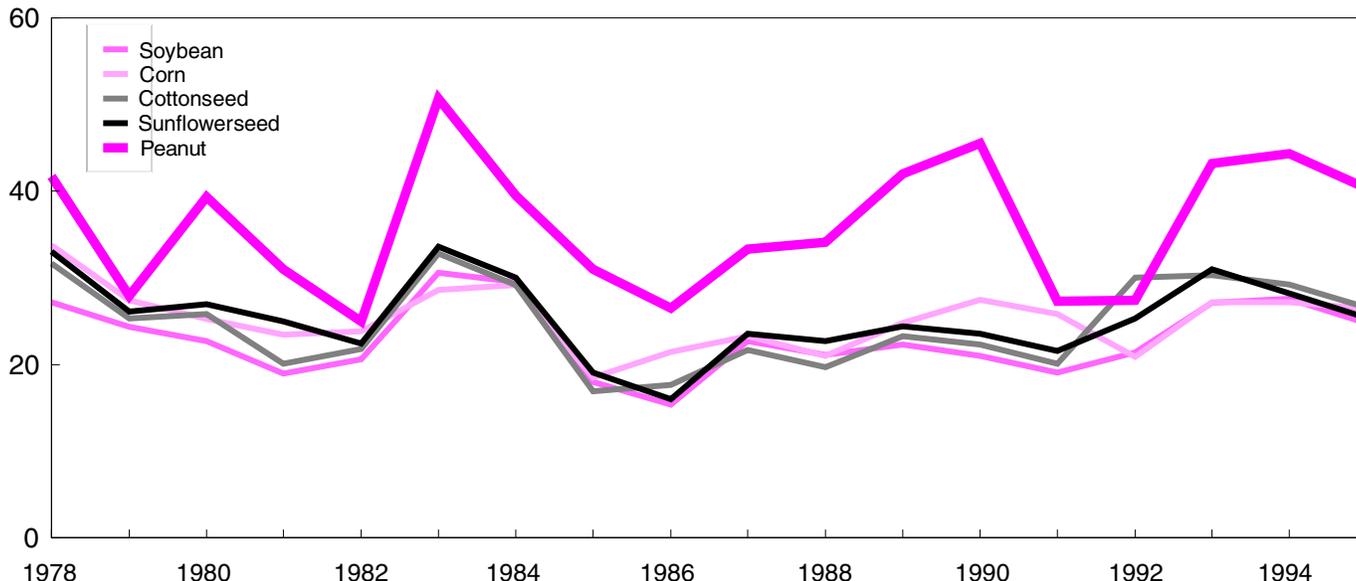
A basic economic problem with using vegetable oils and animal fats for fuel is that these products are more expensive than diesel fuel (fig. 12). The U.S. average refiner price to end users for No. 2 diesel fuel from 1993-95 was \$0.57 per gallon (USDOE, EIA). Since 1978, the closest price margin between diesel fuel and soybean oil occurred in 1981 when soybean oil was priced at about \$1.46 per gallon, \$0.49 higher than the diesel fuel price of \$0.97 (fig. 13). In only two other years, 1985 and 1986, were soybean oil prices lower than \$1.46. Petroleum prices were still relatively high at that time, stemming from the oil crisis that started in the early 1970s and lasting through 1982 (Cambridge Energy Research Associates). The largest gap between the two prices, \$1.72, occurred in 1978. Since 1993, the price spread between soybean oil and No. 2 diesel remained over \$1.50 per gallon. Although lard, tallow, and yellow grease cost less than vegetable oils, they are still priced significantly above diesel fuel. The average price of lard is \$1.33 per gallon, edible tallow \$1.43 per gallon, inedible tallow \$1.04 per gallon, and yellow grease, about \$0.87 (fig. 12).

Figure 11

Prices of selected crop oils, 1978-95

Peanut oil prices have been consistently higher than other oils

Cents per pound



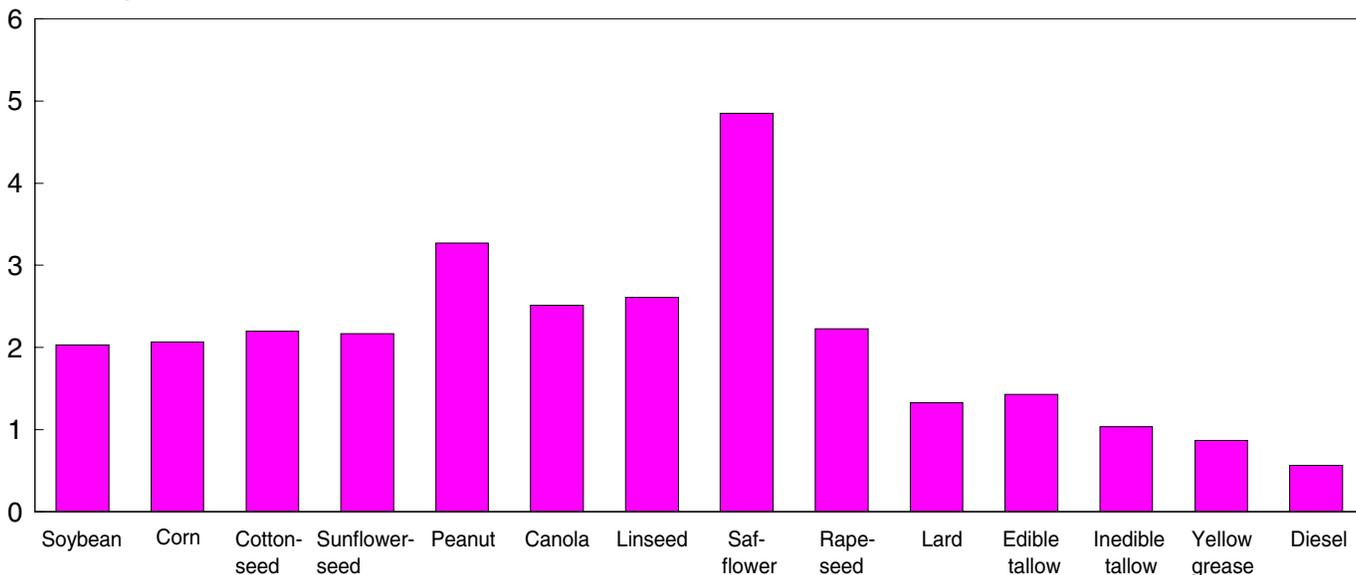
Source: U.S. Department of Agriculture, Economic Research Service, *Oil Crops Yearbook*, October 1996.

Figure 12

Prices of crop oils, animal fats, and diesel fuel, 1993-95

Vegetable oils low in saturated fat tend to cost more while diesel fuel costs the least

Dollar per gallon

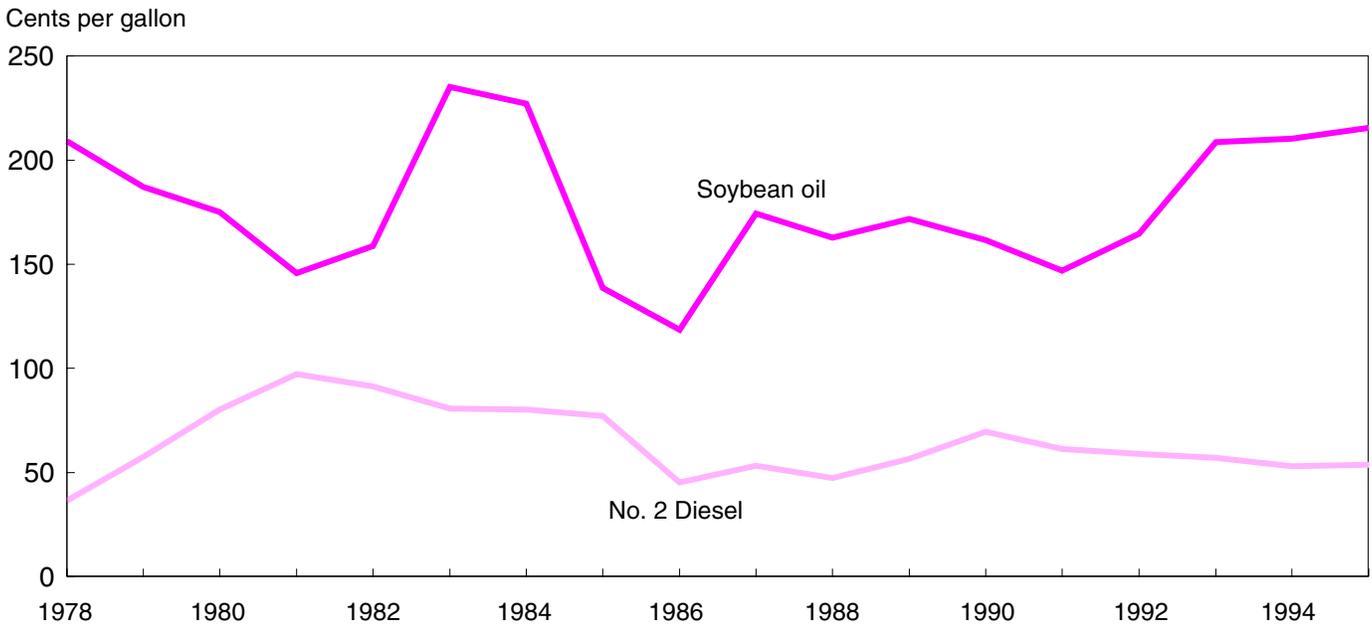


Source: Prices are 3-year average (1993-95) from *Oil Crops Yearbook*, October 1997, USDA, ERS, with the following exceptions: the rapeseed price is a 3-year average (1993-95) Minneapolis trading price from *Industrial Uses of Agricultural Materials Situation and Outlook Report*, USDA, ERS, July, 1997; canola is a 3-year average (1993-95) from *Milling & Baking News*; inedible tallow and yellow grease are a 3-year average (1992-94) from *Feedstuffs, The Weekly Newspaper for Agribusiness*, The Miller Publishing Company, Minnetonka, MN.

Figure 13

Prices of soybean oil and No. 2 diesel

In 1981, soybean oil and diesel fuel prices differed by 49 cents



Source: U.S. Department of Energy, EIA, and U.S. Department of Agriculture, ERS, 1996.

Since the price of diesel fuel includes refiner cost and the agricultural feedstock prices above do not include fuel processing costs, a fair cost comparison between petroleum diesel and biodiesel should include the costs of converting oil into biodiesel less the value of a glycerol coproduct credit. Withers and Noordam reported that the transesterification cost for a 2.3-million-gallon-per-year biodiesel plant was \$0.58 per gallon, plus an estimated total overhead cost of \$0.33 per gallon. They assumed that the plant would produce a technical grade glycerol that generated a net coproduct value of \$0.39 per gallon. This coproduct credit reduces the conversion costs to \$0.52 per gallon. Thus, if a biodiesel producer were to purchase soybean oil at \$2.00 per gallon, the total cost of production would be about \$2.52 for a gallon of biodiesel. If a less expensive feedstock such as refined yellow grease were used, the total cost of biodiesel would be about \$1.39 per gallon, still about \$0.82 higher than the price of petroleum diesel.

Government Regulations Create Potential Niche Markets

Generally, the high price of agricultural oils prevents biodiesel from competing with diesel fuel. However,

higher priced niche markets could develop for biodiesel as a result of environmental regulations contained in the Clean Air Act Amendments of 1990 (CAAA) and the energy security provisions of the Energy Policy Act of 1992 (EPACT). In addition, consumers might voluntarily pay more for cleaner fuels due to perceived environmental and health benefits.

Biodiesel reportedly offers the following benefits relative to petroleum diesel: improved biodegradability; reduced carbon monoxide (CO) and sulfur oxides (SO₂) emissions; reduced odor; reduced particulate emissions in some engines; less soot; and safer handling due to a higher flash point (Peterson et al., 1996; Graboski and McCormick). In addition, the use of biodiesel may reduce some greenhouse gases, oral and dermal toxicity, ozone precursors, and mutagenic and carcinogenic compounds associated with biodiesel exhaust (Peterson et al., 1996; Sheehan et al.). However, more research is needed to fully verify and quantify these potential benefits.

Clean Air Act Amendments of 1990

The CAAA requires the U.S. Environmental Protection Agency (EPA) to identify and regulate air emissions from all significant sources, including on- and off-road

vehicles, urban buses, marine engines, stationary equipment, recreational vehicles, and small engines used for lawn and garden equipment. Two major CAAA fuel emission control programs for motor vehicles, the oxygenated fuels program and the reformulated gasoline program, have already been implemented (Lee and Conway). These programs have increased the demand for alternative fuels such as ethanol and methanol and prospective biodiesel producers hope that, as EPA implements other clean-air programs, new markets will develop for biodiesel and biodiesel blends. The EPA does recognize biodiesel as a means of lowering air pollution in the EPA Urban Bus Retrofit/Rebuild program, which sets particulate matter (PM) standards for pre-1994-model year urban buses in areas with a 1980 population of more than 750,000. EPA began implementing the new PM standards on December 1, 1995. In October 1996, EPA certified B20, used in combination with a catalytic converter, as a PM control device for bus engines in the Urban Bus Retrofit/Rebuild program (*Federal Register*, October 22, 1996). However, it is unlikely that the B20-catalytic converter control package will be widely used by bus operations because other, more economical equipment packages have been certified (USDA, ERS, 1996).

Energy Policy Act of 1992

Another potential market for biodiesel is in EPACT's alternative-fuel motor-fleet program, which is being implemented by the U.S. Department of Energy (DOE). EPACT's alternative-fuel motor-fleet regulations require Federal, State, and alternative-fuel providers to increase their purchases of alternative-fueled vehicles. This program is being phased in over time, with required purchases of alternative-fuel vehicles increasing each year. For example, the Federal fleet's purchases of new light-duty vehicles, starting in FY 1996, must include at least 25 percent alternative-fueled vehicles; 33 percent in FY 1997; 50 percent in FY 1998; and 75 percent in FY 1999 and thereafter. Medium- and heavy-duty vehicles were added to the program in 1996 under Executive Order 13031, which calls on Federal agencies to develop new ways to implement the alternative-fueled vehicle acquisition requirements. The order states that each dedicated alternative-fuel heavy-duty vehicle shall count as three light-duty vehicles.

DOE has designated neat biodiesel as an alternative fuel, but because of its high cost and uncertainty over

full engine-manufacturer warranty coverage when using a non-standard fuel, biodiesel vehicles are not being used by fleet operators (EMA; *Federal Register*, October 22, 1996). The most common alternative-fueled vehicles being purchased use liquefied petroleum gas (propane) and compressed natural gas. Ethanol and methanol vehicles also have been purchased, along with a small number of electric vehicles and vehicles fueled by liquefied natural gas (*Federal Register*, March 14, 1996). Fleet operators may show more interest in biodiesel if lower cost biodiesel blends were an option.

The most common biodiesel blend used today in demonstration and test engines is B20. Some biodiesel advocates are promoting B20 because this blend level significantly reduces particulate matter and is significantly cheaper than 100 percent biodiesel (American Biofuels Association). However, DOE does not recognize biodiesel blends as alternative fuels, thus disqualifying B20 from use in the alternative-fuel program. DOE plans to engage in a separate rule-making proceeding to develop the information needed to consider the addition of any biodiesel blends to their definition of alternative fuel (*Federal Register*, July 15, 1997). In addition, a bill introduced in Congress in late 1997 to amend EPACT includes the use of biodiesel blends in EPACT programs. The Senate Bill "The Biodiesel Energy Development Act of 1997" or S.1141 is pending in the Senate Energy and Natural Resources Committee. The Energy Subcommittee held a hearing on the bill on May 21, 1998. Similar legislation, Bill H.R. 2568, is pending in the House Commerce Committee.

Voluntary Adoption

Consumers may also choose to pay a premium price for biodiesel or biodiesel blends if they perceive these fuels to be cleaner, healthier, and overall better for the environment than petroleum diesel fuel. For example, National Parks could enhance tourism by using biodiesel in tour buses, decreasing odor and lowering visible particulate matter (Haines). Ski areas and other resorts that attract people for their environmental and recreational amenities also might be willing to pay a premium price for biodiesel in their equipment and vehicles. Since biodiesel is non-toxic and much more biodegradable than petroleum diesel, it could become an attractive fuel in environmentally sensitive areas such as wildlife habitats and parklands where off-road vehicles are used. Boat owners in environmentally

sensitive waters, such as the Florida Keys and Maryland's Chesapeake Bay, have shown interest in using this fuel (*Biofuels Update*, 1996). Motor-fleet operators in midwestern States such as Iowa and Nebraska are experimenting with low blends of biodiesel. Not only are State transportation authorities interested in the environmental benefits of biodiesel, they are also interested in creating a potential new market for local soybean farmers. The Nez Perce Tribe Reservation in Idaho is researching biodiesel because the fuel is renewable and environmentally friendly (Cruz et al.).

Government Guidelines

While the environmental and potential health benefits of biodiesel are attractive, it still must meet specific EPA guidelines before it can be sold for widespread use. The CAAA has created opportunities for biodiesel commercialization as well as some regulatory hurdles. For example, manufacturers of all diesel fuels, including biodiesel and biodiesel blends, have to meet CAAA fuel-property definitions and satisfy health-effect requirements. EPA is currently conducting a rule-making process to define a "standard" fuel that meets the Agency's desired emission profile for diesel fuel. Fuel manufacturers will have to determine whether their diesel fuels' chemical compositions are substantially similar to EPA's definition of a "standard" diesel fuel. When the final rule is implemented, biodiesel and other diesel fuel producers must either be able to prove that their fuels are substantially similar to EPA's diesel standard or receive a waiver under CAAA Section 211(f). Since biodiesel is not a petroleum product, it may not fit the strict definition of substantially similar. However, if biodiesel producers can show that their fuels have the same emissions characteristics and the same engine degradation properties as the definition diesel fuel, they may qualify for a waiver. A potential roadblock for producers obtaining such a waiver is that tests results show nitrogen oxide (NO_x) emissions increasing when using biodiesel; NO_x is an air pollutant regulated by EPA (Graboski and McCormick).

Biodiesel producers also must overcome the potential public-health-effect data requirement under CAAA Section 211(b) and (c). These provisions require fuel producers to gather three tiers of research test data on their fuels to evaluate the potentially harmful human-health effects of fuel emissions. Tier I test data were submitted to EPA in April, 1998, and the biodiesel industry is currently preparing tier II testing (*New*

Fuels & Vehicles Report). If these test results are favorable, biodiesel fuels may be exempted from tier III testing.

Feedstock Yields and Fuel Properties

Oil yield varies greatly among potential feedstocks (fig. 14). The supply of the various oils varies significantly from year to year because crops can be affected by adverse weather and other uncertainties (fig. 15). Average oil yield per acre for each U.S. oil crop in the study was estimated by the following steps:

1. An annual oil-yield percentage was estimated by dividing the weight of the annual oil production by the weight of seeds crushed to derive that oil. For example, 1.369 billion bushels or 82.14 billion pounds of soybeans were crushed in 1995, yielding 15.236 billion pounds of oil, which equates to an oil content of 18.5 percent.
2. The oil percentage is multiplied by annual crop yield. In 1995, the yield for U.S. soybeans was 35.3 bushels per acre or 2,118 pounds per acre, so each acre used for oil produced an estimated oil yield of 392 pounds.

A 5-year (1991-95) average oil yield per acre for each oil crop analyzed (fig. 14) was used to account for the annual yield variability in crop production, caused mainly by weather conditions. Peanuts have the highest average oil yield at 764 pounds per acre. Canola, rapeseed, and safflower have oil yields of about 600 pounds per acre. Soybean oil yielded about 400 pounds per acre, and linseed oil about 350 pounds per acre. The oil yield for corn and cottonseed was less than 200 pounds per acre. Over the 5-year period, corn had the highest crop yield variation, ranging from 5,639 to 7,762 pounds of seed per acre; canola had the lowest yield variation, ranging from 1,278 to 1,350 pounds of seed per acre (fig. 15).

Increasing Feedstock Supplies Through Plant Genetics

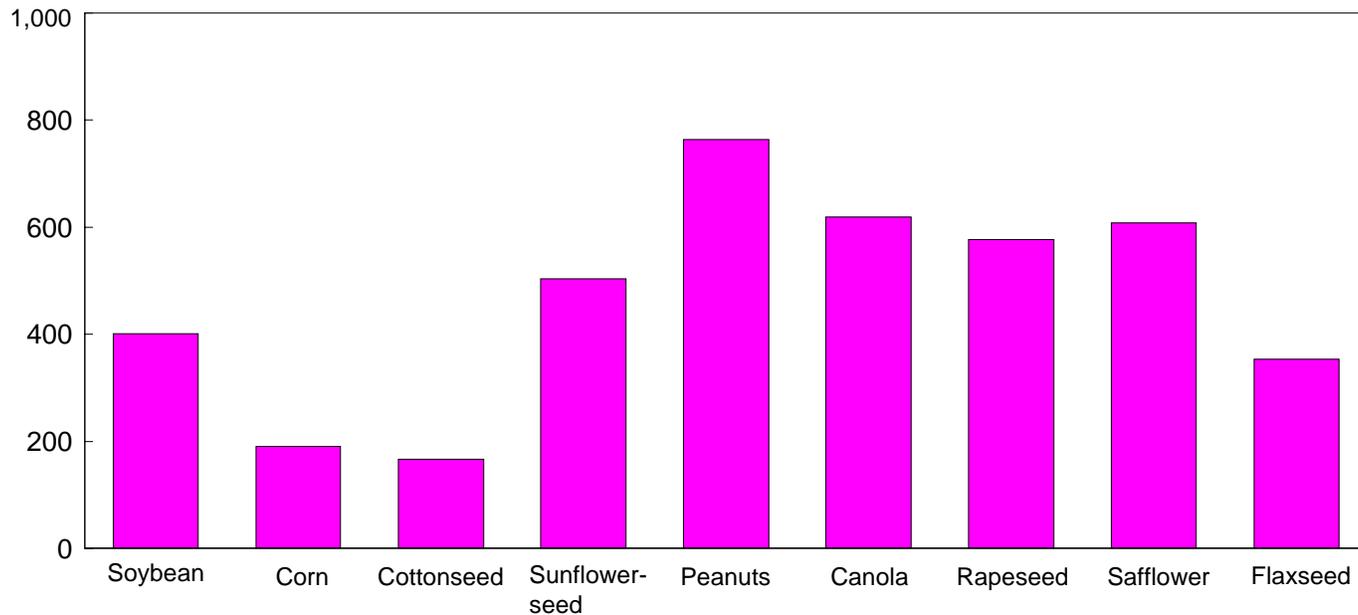
Oil crop yields and the oil content of oilseeds potentially may be increased through genetic modification. Over the past several decades, plant breeders have improved the yields of a wide array of U.S. crops. For example, average corn yield from 1965-69 was 78.5 bushels per acre while the corresponding value from

Figure 14

Oil yields of potential biodiesel feedstocks, 1991-95 average

Peanuts, canola, rapeseed, and safflower have the highest yields

Pounds of oil per acre



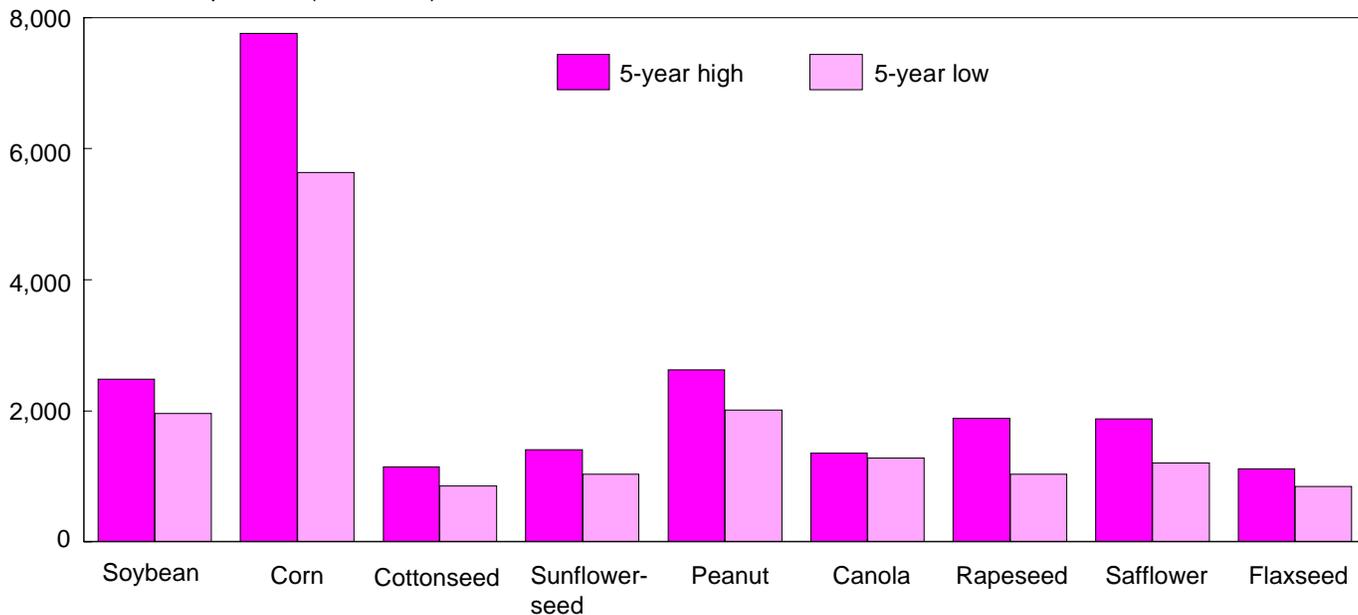
Source: U.S. Department of Agriculture, ERS, *Oil Crops Yearbook*, October 1997.

Figure 15

High and low yield for selected oil crops, 1991-95

Adverse weather and other uncertainties cause annual yield variations

Pounds of oilseed per acre (thousands)



Source: USDA, NASS, *Crop Production Summaries*, 1994, 1995, and 1996.

1990-94 was 119.5 bushels per acre, an increase of about 50 percent. At the same time, soybean yield increased from 25.7 to 36.1 bushels per acre, or by 40 percent. A large part of this increase can be attributed to genetic improvements through plant breeding. Genetic additions also improved resistance to certain diseases and insects. Changes in seed composition also are possible. Oilseed varieties have been developed with an oil content as high as 24 percent of seed weight and protein as much as 52 percent of seed weight. Oil quality may be improved by genes that lower saturated fat content or the levels of certain polyunsaturated fatty acids. These innovations can enhance oilseed value and the nutritional quality of feed grains and food products. They also may play an important role in the development of improved feedstocks for industrial applications, like biodiesel.

The U.S. supply of crop oil could be increased by developing new plant varieties with higher oil content. Although it is possible to increase soybean oil content, it usually results in lower protein, a concern because the demand for protein drives the soybean market; lower protein may actually reduce the total value of the crop. Lowering the availability of high-quality meals could increase meal prices and raise feed costs for livestock producers. Researchers must consider the economic impact of altering the relation between oil and protein content when designing new soybean varieties. Yet, it may be possible to achieve small gains for both protein and oil through genetic modification. Even a small increase in the oil content of some crops, especially soybeans with such large acreage, could make an impact on total U.S. crop oil supply. For example, if the average soybean oil content could be increased by 5 percent, the average annual production of soybean oil could increase by 722 million pounds.

When increasing the protein and oil concentration of the bean, the impact of these changes on yielding ability must be considered. Yield usually is negatively correlated with protein, but positively correlated with oil content. USDA scientists have developed genetic approaches for increasing protein, oil, and yield simultaneously (Wilson et al.). Their strategy involves several lines of attack:

1. The genetic makeup of different soybean varieties is determined to understand why certain soybeans make more oil or protein.

2. The best breeding lines for each trait are identified.
3. Crosses are made to create gene combinations that improve yielding ability in high-protein germplasm.
4. A breeding method is developed that allows for the selection of all three traits.

The USDA Soybean Germplasm Collection contains about 15,000 different accessions of *Glycine max*, the modern cultivated form of soybean, which exhibit a wide range of oil and protein concentrations. However, genetic variation in oil and protein biosynthesis exists, even among different soybeans that have the same phenotype (Burton et al.). As an example, a high-protein phenotype may result from higher protein and normal oil synthetic capacity, or to normal protein and reduced oil synthetic capacity. A case in point, NC-106 and NC-111 are two unrelated germplasm lines that both have 50 percent protein. High protein in NC-106 is due to gene combinations that enable protein synthesis with half the amount of metabolic energy that is required in NC-111. High protein in NC-111 is an indirect function of gene combinations that reduce the ability to make oil. This latter observation appears to be fairly common among high-protein germplasm, and may be the fundamental cause for the inverse relation between protein and oil. Thus, researchers must look for germplasm that inherently exhibits lower apparent energy requirements for these processes while maintaining normal or enhanced synthetic activities. Such traits are a strong criterion for selecting high-protein breeding lines.

Molecular genetic approaches also may help identify germplasm with superior gene combinations for oil or protein synthesis. Genes encode key enzymes in the respective pathways as hybridization probes to determine how well the gene is read or transcribed in a given genotype. A high transcription level for a particular gene suggests greater production of the enzyme or gene-product. Thus, these gene probes may be used as molecular markers to screen germplasm for breeding lines with superior potential for oil and/or protein accumulation (Israel and Burton). As molecular genetic technologies become more useful in soybeans, it may be possible to add more copies of certain genes in these synthetic pathways to create genetic resources that surpass those available in the germplasm collection.

An approach for increasing yielding ability in high-protein, high-oil soybean germplasm may involve genes that determine the size and structure of the plant root

system. While soybeans usually have a large tap root, the plant introduction PI416937 has an extensive fibrous root structure (Pantalone et al., 1996a). Grafts of high-protein germplasm to the root system of PI416937 achieved a 20-percent gain in productivity without affecting protein concentration (Pantalone et al., 1996b), overcoming the negative relation between protein and yield with the proper gene combinations. Addition of genes that determine a fibrous root system to high-protein breeding populations appears to be a relatively easy way to increase yield of high-protein germplasm to levels comparable to current commercial varieties.

This research approach has proven successful with the recent release of the new variety Prolina. This high-protein, high-oil soybean variety was developed using a breeding method that recombines multiple genes associated with protein, oil, and yield in the same population (Burton). This soybean variety achieves 36 percent protein and 19 percent oil, with potential for greater than 50 percent protein meal. The average protein and oil content of commercial soybeans is about 44 and 18.5 percent, respectively. Researchers expect that further work could result in protein and oil concentrations of 46 percent and 22 percent, respectively, in the next few years.

Chemical Composition and Fuel Properties

Among biodiesel feedstocks, composition varies greatly. When used to make biodiesel or biodiesel blends, these feedstocks create fuels with different properties that offer distinct advantages—and disadvantages—over petroleum diesel. The effects to engines must be analyzed to fully compare the potential of biodiesel feedstocks. Seven key fuel properties are examined to determine fuel quality differences between biodiesels and No. 2 diesel fuel (D-2): cold flow, ignition quality, flash point, heat of combustion, viscosity, oxidative stability, and lubricity.

Basic Composition Differences Between Biodiesels and No. 2 Diesel

The most important composition difference between No. 2 diesel and biodiesel is oxygen content. The oxygen content of petroleum diesel is zero, while biodiesel contains 10-12 percent (by weight) oxygen, which lowers energy density and particulate emissions. Also, while biodiesel is essentially sulfur free, on-road D-2 in the United States is allowed to contain up to 500 parts per million (ppm) sulfur as measured by the

American Society for Testing and Materials (ASTM D-2622). In the engine exhaust system, sulfur is converted to sulfur oxides, a fraction of which are converted to sulfuric acid, which then produces fine particulate, a regulated pollutant. Petroleum-derived diesel also contains from 20 to 40 percent (by volume) aromatic compounds, which increase emissions of particulate matter and nitrogen oxides (NO_x). Biodiesel is essentially non-aromatic. Petroleum diesel contains essentially no olefinic bonds, while biodiesel can contain a significant number of these reactive, unsaturated sites that provide pathways for oxidative instability.

Composition of Fatty Acid Chains

Agricultural fats and oils consist of various combinations of fatty acid chains (table 2). The fuel properties of biodiesel will greatly depend on the fatty acid chains of the feedstock used for esterification. All naturally occurring fats and oils are derived by the addition, through esterification, of carboxylic acids (fatty acids) to a single alcohol, glycerol, and are therefore known as triglycerides or triacylglycerols. With few exceptions, the carboxylic acids that are attached (esterified)

Table 2—Melting and boiling points for fatty acid methyl esters found in agricultural fats and oils

Acid chain	No. of carbons	Acid type	Methyl esters	
			Melting point	Boiling point
<i>Degrees Celsius</i>				
Caprylic	8	Saturated	-40	193
Capric	10	Saturated	-18	224
Lauric	12	Saturated	5	262
Myristic	14	Saturated	19	295
Palmitic	16	Saturated	30	338 ¹
Palmitoleic	16	Monounsaturated	0	NA
Stearic	18	Saturated	39	352 ¹
Oleic	18	Monounsaturated	-19	349 ¹
Linoleic	18	Diunsaturated	-35	366 ¹
Linolenic	18	Triunsaturated	NA	NA
Arachidic	20	Saturated	50	NA
Eicosenoic	20	Monounsaturated	-15	NA
Behenic	22	Saturated	54	NA
Erucic	22	Monounsaturated	NA	NA

NA: Not available.

¹Corrected to 760 millimeters by a Cox chart procedure in American Petroleum Institute, *API Technical Data Book*, Petroleum Refining, Figure 5A 1.15, Washington, DC, August 1964. Vacuum distillation data from Aldrich Chemical Company Inc., *Catalog Handbook of Fine Chemicals*, Milwaukee WI, 1994.

to the glycerol at each of its three esterifiable sites are straight-chain compounds ranging in size from 8 to 22 carbons. For most fats and oils of interest, the majority of the fatty acids have 16 and 18 carbon-length chains. For example, beef tallow is composed of about 54 percent saturated fat, which primarily is in the form of palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid. Most of the major crop oils such as soybean, corn, and cottonseed contain predominantly unsaturated acids, the majority being oleic (mono-unsaturated) and linoleic (doubly unsaturated) acids. Rapeseed oil from some sources contains a high percentage of the monounsaturated C22 erucic acid.

The chemical composition of fat and oil esters depends on the length and degree of unsaturation of the fatty acid alkyl chains. Fatty acids, found in the most common biodiesel source materials, have an even number of carbons, and may be saturated (no olefin bonds) or unsaturated (one or more double bonds) (table 2). An unsaturated fat may be converted to a saturated fatty acid by a chemical process termed hydrogenation.

Freezing and Boiling Points

Freezing point (or melting point) is the temperature or temperature range in which a pure compound or mixture freezes. Pure compounds freeze at a specific temperature, while mixtures freeze over a range. D-2 has a freezing point range that can be made to suit most climates, with refiners producing various formulations to match the different weather conditions experienced throughout the country. Biodiesels generally have higher freezing point ranges than D-2. Saturated esters exhibit higher freezing points than unsaturated esters (table 2). Stearic acid, for example, is solid to 39°C, while methyl oleic ester (methyl oleate) melts at -19°C and methyl linoleate at -35°C. The only difference is the presence of a single double bond in the structure of oleic acid and two double bonds in the linoleic acid structure. The use of biodiesel fuels with higher freezing points may result in fuel system problems in very cold temperatures.

Boiling point is the temperature at which a fuel boils. Mixtures boil over a range of temperature—for fuels, the range is specified from the initial boiling point to the final boiling point. While the freezing point depends on chemical structure, the boiling points of the esters depend on the length of the carbon chain and are nearly independent of the degree of unsaturation of the fatty ester. As mixtures of a few relatively similar

compounds, fat and oil ester fuels have a narrow boiling range relative to D-2. Average boiling points for various biodiesels range between 325-350°C (Graboski and McCormick), which is near the high end of the range reported for diesel, an indication that biodiesel boils at a temperature near the maximum allowed by ASTM for D-2. Fuels with distillation characteristics different from D-2 will probably also have different emissions profiles. However, as biodiesel is blended with D-2, the differences between the fuels are less significant and tests show that B20 can meet the ASTM distillation specification.

Cold Flow

Cold flow properties measure a fuel's ability to function in cold temperature. With higher freezing points, it follows that biodiesels would have less desirable cold flow properties than D-2. The key temperature-flow properties for winter fuel specification are cloud and pour points, which describe the freezing range of a fuel. Cloud point (ASTM D-2500) is the temperature at which, as the fuel is cooled, wax that may plug the fuel filter begins to form. It is measured as the temperature of first formation of wax as the fuel is cooled. Pour point (ASTM D-97), a measure of the fuel gelling point, is the temperature at which the fuel is no longer pumpable. The pour point is always lower than the cloud point. Fuel cloud and pour points are often varied by refiners to meet local climatic conditions. During winter months, chemicals are added to D-2 to modify the size of the wax crystals and reduce the pour point. Also, D-2 can be diluted with No. 1 diesel fuel or kerosene to meet wintertime flow specifications.

Midwest Biofuels reported that cloud and pour points for methyl esters of soybean oil, rapeseed oil, and tallow were relatively high compared with winter ambient temperatures. Freezing points of organic molecules are very sensitive to chemical structure. The structural properties of biodiesel that affect freezing point are degree of unsaturation, chain length, and degree of branching. Tallow methyl esters (methyl tallowate) are poorer in this respect than soybean and rape methyl esters because of the higher degree of saturation and higher freezing point of the ester. Methyl soybean and rapeseed esters exhibit average cloud points of about 0°C and -5°C and pour points of about -4°C and -10°C, respectively. Methyl tallowate exhibits an average cloud point of 14°C and a pour point of 10°C.

Blending biodiesel with petroleum diesel at the 20-percent level results in intermediate cold flow properties. The Midwest Biofuels study suggested that the higher cloud points of methyl esters may not be sufficiently repressed by No. 1 or No. 2 diesel to produce a fuel that will not plug fuel filters in the winter. Further diluting and lowering the biodiesel blend (e.g., B15 or B10) may lower the pour point sufficiently to minimize gelling problems, but research has not adequately addressed this question.

Cold flow or flow-improver additives can improve the cold flow properties of diesel fuels, and researchers have tested their effectiveness in biodiesel (Dunn et al.). These additives do not prevent beginning wax formation (that is, do not change the cloud point) but keep the small wax crystals from growing and combining. Thus the additives improve pour point and cold filter plugging point (CFPP), the point at which wax crystals can stop fuel flow by clogging the engine fuel filter. CFPP is usually below the cloud point and above the pour point. The Midwest Biofuels study of the effect of pour point depressants showed that gelling could be eliminated with appropriate commercial additives. The pour point of soybean methyl ester was reduced to -40°C with a commercial additive at 1,000 ppm, levels low enough for successful commercial use. However, the results of the study did not clearly show a reduction in CFPP even with high additive concentrations. The cold flow properties of biodiesel fuels are clearly an area in need of more research. Development of new flow-improver additives specifically for fatty acid esters may be a fruitful area because we expect that flow-improver additives will be necessary for consumer acceptance of biodiesel fuels and blends, especially for winter use. Research should be aimed at developing ways to utilize fatty acids with superior cold flow properties. For example, Harrington showed that high levels of palmitic and stearic acid esters from oilseeds greatly increase the cloud point much more than do oleic acid esters alone. Modified or reduced palmitic and stearic acid esters and optimized oleic acid esters might therefore improve cold flow properties.

Cetane Number and Ignition Quality

The cetane number of a fuel, specified by ASTM D-613, is a measure of the fuel's ignition delay. Higher cetane numbers indicate shorter time between the beginning of fuel injection into the cylinder and ignition, a desirable property in diesel engine fuel. Increasing the cetane number may reduce NO_x emis-

sions but, above 55-60, higher cetane numbers have little additional positive effect on emission reductions (Heywood). D-2's cetane numbers range from 40 to 52, while numbers for biodiesels depend on the parent oil source but generally measure at the high end of the range of No. 2 diesel. The cetane numbers of diesel fuels and biodiesels can be enhanced using chemical additives. Some of these additives may also help reduce the increase in NO_x emissions usually observed with biodiesel and biodiesel blends (Graboski et al.). However, Clark et al. showed that the NO_x did not decline when neat biodiesel was treated with ethylhexylnitrate, a widely used cetane improver in low-quality diesel fuels. Sharp's tests on B20 gave similar results, but using a different cetane improver, di-*t*-butyl peroxide, NO_x emissions did decline.

Reported cetane numbers range from about 44 to 57 for soybean oil and 48 to 61.8 for rapeseed oil methyl esters. This broad range of values may be caused by differences in the fractional conversion of triglycerides to esters and by the presence of residual methanol and glycerol in the fuel or by oxidation of the esters, which results in the formation of hydro peroxides that increase the cetane number (Van Gerpen, 1996). Examination of flash point and distillation data supports this conclusion for biodiesels measuring cetane numbers below about 50. The average cetane number from various test results is 50.9 for soybean methyl esters and 52.9 for rapeseed methyl esters. Cetane numbers for other esters reported in the literature range between 48 and 60. Highly saturated esters such as those prepared from tallow are generally expected to have the highest cetane numbers (Graboski and McCormick).

Flash Point

Flash point (ASTM D-93) measures the temperature to which a fuel must be heated so that the mixture of vapor and air above the fuel can ignite. The higher the flash point, the less likely a fuel will ignite accidentally. All number-2 diesel fuels have relatively high flash points (54°C minimum, 71°C typical) and are considered to be safe fuels under normal conditions. Biodiesels are even safer, with flash points typically greater than 100°C . A review of recent studies indicates that the average flash point of methyl esters ranges from 117°C for tallow to 170°C for rapeseed (Graboski and McCormick). The average flash point for soybean oil methyl ester is about 131°C . However, since purity is uncertain, it is difficult to compare the flash points of esters from various sources. Since

material with a 90°C or greater flash point is considered non-hazardous from a storage and fire-hazard point of view under U.S. Department of Transportation regulations, neat biodiesel is much safer than petroleum diesel. In blends, the petroleum diesel flash point will prevail. The Engine Manufacturers Association has expressed concern that because of the oxidative instability of soybean and tallow esters, flash points (as well as other properties discussed below) may change during storage. This possibility appears not to have been investigated in quantitative studies.

Heat of Combustion

On a volumetric basis, biodiesels contain slightly less energy than D-2, i.e., fuel economy is slightly less. Number-2 diesel exhibits a specific gravity of 0.85 (ASTM D-287). Biodiesel's specific gravity, reported to vary between 0.86 and 0.90, is typically 0.88. Therefore, volumetric metering of biodiesel (as in the unit injectors used in modern diesel engines) results in the delivery of a slightly greater mass of fuel. Biodiesels also have a lower energy content on both a volumetric and a mass basis. So, although a larger mass of fuel might be delivered by a unit injector, the actual energy delivered is less than with number-2 diesel. It is generally accepted that fuel consumption is proportional to the volumetric energy density of the fuel based on the lower or net heating value. On a lower heating-value basis, number-2 diesel contains typically 132,000 Btu/gal while biodiesels contain approximately 10 percent less, e.g., soybean methyl ester has a lower heating value of 117,400 Btu/gal. Thus the volumetric fuel economy will be lower for biodiesel and biodiesel blends, i.e., at a given volume, biodiesel has a lower mileage range (Graboski and McCormick). However, as Graboski and McCormick have shown, the energy efficiency on a lower heating value Btu basis is about the same for diesel fuel, biodiesel, and biodiesel/diesel blends.

Viscosity

Viscosity measures the thickness of a fuel; high viscosity leads to poorer atomization of the fuel spray from the fuel injectors. Biodiesels have higher viscosity than petroleum diesels. The ASTM D-445 specification of maximum 4.1 cs viscosity at 40°C is barely met by soybean methyl esters. Viscosity values for other neat biodiesels exceed this value significantly and are higher than for typical number-2 diesel (Graboski and McCormick). Average viscosity levels of biodiesels reported in the literature are 4.08 for soybean oil methyl ester, 4.83 for rapeseed methyl ester, and 4.80

for tallow methyl ester. Additionally, as temperature is decreased, the viscosity of biodiesel and biodiesel blends increases more rapidly than that of D-2 (Van Gerpen). However, the effect of biodiesel content on the blend viscosity is lower than predicted by a linear combination model and it appears that blends containing up to 30 weight percent of soybean or rapeseed methyl esters will most likely meet viscosity standards for number-2 diesel.

Iodine Number and Fuel Oxidative Stability

Iodine value (IV), a measure of the degree of chemical unsaturation of the fuel, is found by reacting the fuel with iodine, which saturates all the olefinic bonds. A fuel's iodine number is the amount (grams) of iodine required to fully saturate the olefinic double bonds in a sample of 100 grams; a high value represents a high degree of unsaturation, which can lead to deposit formation and storage-stability problems with fuels. Schafer suggests that fuels with iodine numbers above 115 are unacceptable. Ryan et al., looked at soybean, sunflowerseed, and cottonseed oil methyl ester use in engines and suggest that the maximum iodine number should be limited to 135. Number-2 diesel typically has an iodine number of less than 10. Soybean methyl ester has a relatively high IV of 133 and rapeseed methyl ester has an iodine number of 97. Iodine numbers for other esters reported include 126 for sunflower oil and 106 for cottonseed oil. Peterson et al. reported numbers for methyl and ethyl tallowate at 49 and 47, respectively. Foglia reported that iodine numbers for methyl tallowate ranged from 50 to 60.

Fuels with high iodine numbers may have oxidation problems. Fuel oxidation can decrease fuel storage life and contribute to deposit formation in tanks, fuel systems, and filters. The Engine Manufacturers Association (EMA) reported that compared with number-2 diesel, tallow and soybean oil methyl esters were far more prone to oxidation. Number-2 diesel consumed 5 percent of the available oxygen, tallow methyl esters consumed 60 percent, and soybean oil methyl esters consumed 90 percent. Clearly, soybean-oil-based biodiesel fuels exhibit poor stability relative to conventional diesel, and even though rapeseed-based biodiesel has a significantly lower IV than soybean-oil-based biodiesel, its iodine number is still higher than number-2 diesel. The occurrence of changes in rapeseed-based biodiesel during 2 years of storage was demonstrated by Peterson et al. (1983). The changes slightly reduced engine performance in short-term tests. In addition,

fuels with high iodine numbers may possess high gum numbers, which is a measure of deposit formation. Clark and coworkers determined gum numbers for methyl and ethyl esters of soybean oil and found them to be 16,400 and 19,200, respectively. A comparable value for number-2 diesel is 6 to 7. Biodiesel feedstocks that are naturally highly unsaturated can be treated by hydrogenation, but this leads to a worsening of cold flow properties. Thus, research should be directed at developing additives that can act to stabilize the unsaturated bonds in biodiesel fuels to obtain an acceptable stability without harming cold flow properties.

Fuel Lubricity

Lubricity is an important fuel property because it can help reduce engine wear. Fuel lubricity is critical in diesel engines because in fuel pumps the moving parts are actually lubricated by the diesel fuel itself. Biodiesel generally has higher lubricity than on-road diesel fuel, especially for diesel fuels that have been modified to reduce their sulfur content. Although the lubricity levels of commercial diesel fuels are generally acceptable, increased lubricity may yield further benefits. A Premium Diesel Task Force set up in 1996 by the National Conference on Weights and Measures (NCWM), with support from the American Society for Testing and Materials (ASTM), is analyzing the merits of using enhanced-lubricity fuels in diesel engines. The objective of the Task Force is to determine what fuel properties distinguish a “premium diesel” fuel from a standard diesel fuel and how this fuel should be labeled at the pump to facilitate consumer preference. A lubricity additive is being considered as one component of a “premium diesel” formulation that may also contain additives that will enhance a diesel fuel’s cetane value, energy content, and cold flow properties.

Lubricity is measured by the Scuffing Load Ball On Cylinder Lubricity Evaluator (SLBOCLE) and the High Frequency Reciprocating Rig (HFRR) tests. The National Biodiesel Board has sponsored tests to measure the lubricity of biodiesel and biodiesel blends made from soybean oil (Howell). In addition, Sharp (1996) conducted lubricity tests on rapeseed methyl and ethyl esters. Generally, lubricity tests have shown that biodiesel has superior lubricity when compared to conventional low-sulfur diesels. Furthermore, blending biodiesel with low-sulfur diesel fuel at concentrations of 0.5 percent or higher results in a measurable improvement in lubricity.

Summary

No particular feedstock appears to have an absolute compositional advantage or disadvantage nor does any rank superior to D-2 in all areas. Highly saturated feedstocks, such as tallow and lard, have a disadvantage because they have higher freezing points. On the other hand, these highly saturated esters have the highest cetane numbers, which helps reduce NO_x emissions. Biodiesels have higher viscosity values than D-2, which appears to be a problem regardless of the feedstock source. Soybean methyl ester has a high iodine number, indicating storage stability problems. Rapeseed methyl ester and tallow esters have lower iodine numbers relative to soybean methyl ester because of their high degree of saturation. However, compared with D-2, all biodiesels have high iodine numbers and gum numbers, which can shorten fuel storage life and contribute to deposit formation in fuel storage tanks. In general, biodiesel feedstocks and biodiesel blends have higher lubricity than D-2. Some biodiesel fuel properties can be improved with additives such as cetane enhancers and cold flow improvers. However, more research is needed on developing additives and improving biodiesel’s effect on NO_x emissions, viscosity, and oxidative instability to help biodiesel become a commercially acceptable fuel.

Genetic Engineering May Improve Feedstocks

The ideal biodiesel would combine all the desirable qualities from all of the feedstocks. It may be possible to genetically alter certain attributes of oilseed composition to achieve an ideal base stock for biodiesel. Genetic modification of oilseed fatty-acid composition is no longer an abstract idea; the potential of this technology is just being realized, although most efforts have been directed toward edible products. Using this technology to enhance feedstocks through genetic engineering could possibly solve some of the physical and emissions problems of biodiesel, including ignition quality, NO_x emissions, cold flow properties, and oxidative stability.

Improving Cetane Numbers

Ignition quality and NO_x emissions both are a function of cetane number; a high cetane number is very important for cold-starting diesel engines. In addition, diesel engine emissions of particulate matter and NO_x show improvement with increased cetane num-

ber, up to 55-60. Commercial cetane additives have achieved only limited success in reducing NO_x in biodiesels (Graboski et al.). Thus, increasing the cetane number of biodiesel feedstocks through genetic engineering may be a practical approach for improving ignition quality while simultaneously addressing biodiesel's NO_x problem.

Cetane values specifically for fatty acid methyl esters (FAME) have been estimated as low as 44 for esters made from oilseed crops with common genetic traits (Krisnangkura). A number of soybean breeding lines have been developed with altered fatty acid composition, but the cetane values of vegetable oils from these genetically modified crops have not been reported in the biodiesel literature. The various phenotypes of these engineered plants exhibit maximum fatty-acid content of 27 percent palmitic acid, 28 percent stearic acid, 80 percent oleic acid, 70 percent linoleic acid, and 14 percent linolenic acid. (Normal soybean oil contains 12 percent palmitic acid, 4 percent stearic acid, 21 percent oleic acid, 55 percent linoleic acid and 8 percent linolenic acid.) Some of these altered fatty-acid compositions may improve the cetane value of soybean-based biodiesel. Plant breeders and molecular geneticists need an efficient means to estimate cetane values for modified vegetable oils to help target the most advantageous gene systems and effectively redesign vegetable oil for biodiesel.

The type of fatty acid composition needed to improve ignition characteristics may be approximated from cetane indices designed for fatty acid methyl esters (Krisnangkura). One such cetane index for fatty acid methyl esters parallels the cetane index used for petroleum diesel. This method probably is inadequate, but may give reasonable initial estimates. As an example, calculations based on the composition of methyl esters from various genetically modified crude oils suggested no correlation between palmitic-acid or linolenic-acid concentration and cetane (Krisnangkura); the best overall cetane correlations were found with oleic and linoleic acids. A very strong positive relationship was found between the calculated cetane index of crude oil and oleic-acid concentration; an equally strong negative correlation was estimated with linoleic acid. These two indices suggest that gene systems that affect the enzymatic conversion of oleic acid to linoleic acid can be used to decrease this conversion rate and enhance the cetane index. Also, since there is essentially no correlation between conversion of linoleic to linolenic acid and the cetane index, it would be desirable to increase

this conversion process. This would replace linoleic acid, which reduces cetane, with linolenic acid, which has a neutral effect on cetane.

Understanding how a plant makes oil has helped scientists identify the enzyme activities associated with fatty acids in the creation of triacylglycerol. Triacylglycerol biosynthesis, the pathway of seed oil production, maps out 12 enzyme-reaction steps that an oil plant follows to form triacylglycerol. Enzymes catalyze specific biochemical reactions at each of the 12 steps (fig. A in appendix). For example, the reaction that converts oleic to linoleic acid is found at step 9 and the reaction that converts linoleic to linolenic acid is found at step 10 in the glycerolipid synthetic pathway (fig. A).

Several soybean genes encode enzymes that convert oleic acid to linoleic acid. These genes are found in different plant organs (leaves, seeds, and roots) and in different cell organelles (microsomes, plastids). The predominate gene that governs this reaction in soybean seed has been cloned (Kinney). Natural (recessive allele) or molecular genetic modifications (antisense orientation) to this gene reduce enzyme activity and increase oleic acid concentration from about 20 to 80 percent of total oil. Another means of reducing linoleic acid is by adding a wild soybean gene to a cultivated soybean, which reduces synthesis of linoleic acid and results in higher linolenic acid concentration (Pantalone et al., 1997).

Oleic-acid concentration can also be increased by various plant-breeding or molecular-genetic approaches that can redirect lipid metabolism. In addition to changes in the gene that governs conversion of oleic to linoleic acid, recent evidence shows that antisense (backwards) insertion of a gene at step 8 (fig. A), which governs phosphatidylcholine synthesis, also results in higher oleic-acid concentration (Dewey et al.). As knowledge and ability to combine several desirable gene systems improve, it will become possible to develop soybean genotypes with very high oleic concentrations (table 3). The 'anti-Fad2' genotype, achieved through molecular genetic technologies, has oil with almost 80 percent oleic acid, low iodine value, and significantly greater calculated cetane index. The germplasm 'N96-1106', selected from conventional plant breeding populations, exhibits a high estimated cetane value.

Cetane value can also be affected by changes in the type of mono-unsaturated C18 fatty acid present in

Table 3—Genetic traits for improved cetane index of soybean oil methyl esters

Genotype	Carbon number					Iodine value	Calculated cetane index
	16:0	18:0	18:1	18:2	18:3		
	<i>Percent total fatty acid</i>						<i>Percent</i>
Anti-Fad2	9	3	79	3	6	88	55
N96-1106	7	4	69	18	2	95	54
C1943	4	4	31	55	7	138	44
Normal	11	3	20	58	8	137	44
A6	7	26	26	36	5	96	53
LSD $\alpha = 0.05$	2	6	17	15	2	16	4

Notes: Genotypic mean for heat of combustion: 2805 ± 5 . Genotypic mean for saponification number: 190.2 ± 0.6 .

soybean oil because the position of the unsaturated bond influences the cetane value of pure methyl esters. When the unsaturated bond occurs at the ninth carbon from the carboxymethyl ester group, the fatty acid created is oleic acid with a cetane value of about 47. Moving the unsaturated bond to the eleventh carbon creates vaccenic acid with a cetane value of about 49. Moving the unsaturated bond back to the sixth carbon creates petroselenic acid and a cetane value of about 55. Genes for enzymes that catalyze vaccenic and petroselenic acid synthesis have been cloned from other plant species and may be used to replace the gene that converts oleic to linoleic acid in soybeans. While a few laboratories are experimenting with these modifications, time is needed to see how replacement of oleic with vaccenic or petroselenic acid affects ignition of biodiesel. These changes in fatty acid composition are not expected to affect heat of combustion or saponification number, which is an indicator of quantity, of these alternate fuels.

High-stearic acid concentration also may be a useful trait for enhancing the cetane index of methyl esters from soybean oil. High-stearic acid oils such as the genotype A6 (table 3) have been developed for food products. If a high-stearic acid oil is acceptable for biodiesel, the probable attack point may be the genes that encode the enzyme at step 2 (fig. A). Reducing this enzyme's activity results in higher concentrations of stearic acid.

Improving Cold Flow Properties

Development of new varieties of soybeans could focus on improving cold flow properties; the new low-palmitic acid soybeans, such as the genotype C1943 (table 3), may offer such hope for biofuels (Lee et al.).

At least two genes that determine low-palmitic acid concentration in soybeans are believed to encode different enzymes at steps 3 and 4 (fig. A). Reducing the enzymes' activities lowers palmitic-acid concentration. However, a different way to lower palmitic and increase oleic has been demonstrated by the novel molecular genetic insertion of a mammalian gene that converts palmitic and stearic to monounsaturated fatty acids (Moon et al., 1997). This molecular genetic alteration redirects lipid metabolism at step 5 (fig. A).

Another genetic modification in saturated fatty acid composition that was used by Calgene, Inc. to develop high-laurate (C12) canola may also create oils that produce FAME with lower boiling points (Voelker et al.; Dehesh et al.). Based on research with *Cuphea* species and California Bay Laurel, which are naturally high in lauric acid, Calgene cloned genes that encode thioesterase enzymes that short circuit fatty-acid synthesis at the metabolic step that occurs directly before production of palmitic acid. Expression of these genes in transgenic canola resulted in lines that had about 39 percent lauric acid. Continued advances in molecular genetic technologies will likely make it possible to insert these genes into soybeans to establish high-lauric soybean varieties, which may be used to create a biodiesel with a low boiling point and improved cold-start ability.

Improving Oxidative Stability

Oxidative stability is a measure of the fuel's ability to absorb and react with free oxygen in the atmosphere to produce gums and precipitates that can plug, foul, and corrode fuel tanks and fuel systems. Oxidative instability correlates with high iodine values (degree of unsaturation of a fuel) and more olefinic bonds are thought to

provide the pathway for oxidation. Thus, it appears that saturating the olefinic bonds would stabilize conventional biodiesels. The 'anti-FAD2' and the N96-1106 genotypes mentioned above have relatively low iodine values and possess the genetic trait that significantly improves the stability of soybean oil. Although these genotypes were designed to increase the length of time that non-hydrogenated oils may be used in food applications, they also could enhance oil quality for long-term storage of biodiesel. However, lowering unsaturation could lead to worse cold flow properties.

Balancing Genetic Engineering

A balanced research approach is needed that simultaneously addresses ignition quality, NO_x emissions, cold flow, and the oxidative stability of biodiesel. Researchers could concentrate on two different types of feedstock oil:

- The first fuel, a single oil that would maximize overall fuel performance, would perform well under most conditions but, similar to D-2, may require cold weather treatment in extremely cold weather. This fuel would be developed by coupling all the genes that enhance biodiesel properties into a single genotype. It would have a relatively high percentage of oleic acid combined with low levels of palmitic, linolenic, and stearic acids. Soybean germplasm with these oil traits are scheduled for release in the public domain in 1999. These genetic modifications could boost the cetane index about 20 percent, lower iodine value about 37 percent, and improve cold flow properties.
- The second fuel, similar to the first, would exhibit a much higher level of stearic acid. Aimed at maximizing cetane number at the expense of cold flow, this oil would make an ideal biodiesel for warmer seasons or the southern United States. Soybean germplasm with these traits are scheduled for release for variety development in 2000.

Biotech approaches can modify vegetable oil plants to become ideal feedstocks for biodiesel. Plant breeding and molecular genetic technologies may alter the fatty acid composition of methyl esters to achieve desirable properties. Soybean germplasm with gene combinations enable a higher degree of saturation to improve oxidative stability, ignition quality, and NO_x emissions. In addition, manipulating other genes can improve the cold flow properties of biodiesel. In the near term, these genetic traits may be combined in

agronomic varieties for U.S. production. In the future, adding new genes to the soybean could further increase saturation and alter chain length. The nature of these changes in fatty acid composition would enhance the use of soybean oil for biodiesel as well as improve soybean oil quality in many edible products.

Conclusion

While many oilseed crops and animal fats are suitable feedstocks for biodiesel, the Nation's total supply of these commodities is small relative to total fuel consumption, and these commodities generally receive a higher price as food products. Higher priced niche markets created by environmental regulations could develop for biodiesel because of its cleaner emission benefits, but these benefits have not yet been fully quantified. Also, biodiesel would face stiff competition in these markets from other alternative fuels, such as natural gas.

Given the economic conditions of the 1990s, market opportunities for biodiesel will be limited in the short run. Producers will likely concentrate their marketing efforts on biodiesel blends because blends are much cheaper than neat biodiesel and can still significantly reduce some emissions relative to petroleum diesel. Also, to keep costs down, biodiesel producers are likely to use the least expensive feedstocks available, such as yellow grease. Thus, the commodity markets for soybeans and other oil crops will probably not be affected by biodiesel demand in the near future.

Research Recommendations

In the long run, a focused research program is necessary to create a sustainable market for biodiesel. Long-term research activities need to be directed toward four major areas:

1. Helping biodiesel become more cost competitive;
2. Improving the quality of biodiesel by optimizing its fuel properties;
3. Quantifying and fully understanding the nature of biodiesel emissions from diesel engines; and
4. Estimating the economic effects of developing a biodiesel industry on U.S. agriculture.

Producers could become more competitive and expand their markets by developing a transesterification process that produces multiple products. With additional refining steps, a number of high-valued coproducts may be produced along with biodiesel, such as glycerine, caproic acid, and propionic acid. Research is needed to investigate the technical feasibility of designing a multi-product refinery for fatty acids capable of producing substitutes for diesel fuel, petrochemicals, and other products. In addition, an economic analysis should be conducted to estimate the joint profitability of marketing multiple products, including a price-competitive biodiesel for the diesel fuel market.

Another potentially rewarding research activity would be to investigate biodiesel's market potential as a diesel fuel lubricity additive. Including biodiesel as a lubricity component in a "premium diesel" may soon be approved by the Premium Diesel Task Force, which could increase the demand for lubricity additives and create an additional market for biodiesel. Interest in this potential market has already led to the development of a lubricity additive called "SoyGold," which is being sold by Ag Processors Inc., a soybean methyl ester producer.

Using biodiesel as a fuel additive may be more economical than B-20 and less agricultural feedstocks would be needed to sustain a sizeable market. In addition, EPA requirements such as health effects testing and "sub-sim" may not strictly apply to a biodiesel additive. Such an additive would probably be 1-2 percent biodiesel by volume blended with petroleum diesel. Even this low biodiesel level could enhance fuel performance because of biodiesel's high lubricity level, but more research is needed to quantify the value of using lubricity additives in standard diesel fuels. In addition, the market should be analyzed to identify the diesel fuel additive products currently on the market and to compare their prices and performance claims relative to biodiesel.

The environmental and health benefits of biodiesel and biodiesel blends should be fully quantified. The non-market environmental and health benefits of biodiesel, such as the public welfare benefits of using a completely degradable and nontoxic biodiesel on government lands, could offset the higher cost of biodiesel. As a part of this research, a cost/benefit analysis could be conducted to examine the tradeoff of increased fuel costs with the cost of air emissions and mitigating environmental cleanup costs associated with spills.

The benefits and costs of using biodiesel in order to reduce emissions of global warming gases also need to be explored.

Biodiesel could also become more competitive by expanding and improving its feedstock base through plant breeding and molecular genetic technologies. Scientists are capable of developing soybeans and other crops that produce both higher oil content and different oil properties—maybe even an oil crop with qualities designed specifically for biodiesel production. Detailing feasible research targets could focus future work; researchers should also consider whether genetic changes would alter the nature and quality of the meal produced. Such research should be done in concert with an economic study to estimate the effect of oil crop modifications on agricultural commodity markets.

In order to capitalize on the environmental benefits of biodiesel, more scientific research is necessary to fully quantify the emissions of biodiesel and biodiesel blends in diesel engines. Studies that suggest that oxygenates like biodiesel tend to increase NO_x emissions when used in diesel engines should be expanded to show why oxygenates increase NO_x and how biodiesel fuel properties affect NO_x . Such research could involve fundamental combustion mechanistic studies using sophisticated computer simulation techniques on diesel engines designed to allow the emissions in the flame to be probed in real time.

The toxicities of biodiesel gaseous and particulate emissions should be established. EPA categorized diesel smoke as a Class B carcinogen, likely linked to cancer. Biodiesel emissions are chemically different from diesel; they contain significantly lower polyaromatic concentrations but more aldehydes. The status of health effects studies related to biodiesel needs to be assessed and research gaps identified. It is particularly important that EPA receive all the public health effect information and data required under the CAAA. Positive findings from this information could enhance biodiesel's credibility as an environmentally preferable fuel.

Finally, if a sustainable market for biodiesel is to be developed, it is essential to determine the effect such a market would have on current agricultural commodity markets. The dynamic effect on the agricultural sector from increasing the demand for agricultural oils as fuel should be fully investigated. Researchers should determine: how much biodiesel would have to be produced to significantly increase soybean prices and

raise farm income; the effect of increasing soybean production on the supply of soybean meal and the costs of livestock feed; the effects of using agricultural commodities for fuel on consumer food prices; and the effect of using rendered products for biodiesel on lard, tallow, and yellow grease prices, on soybean demand, and on the feed costs of livestock producers. These questions should be addressed before designing research and policy strategies to promote the production of biodiesel. Furthermore, competition for using

agricultural land to grow energy feedstocks could intensify in the near future—oilseed crops grown for biodiesel, biomass grown for ethanol and biopower, and other new uses for agricultural products could increase farmland demand significantly. An economic analysis will help answer the questions of how much biodiesel and other biofuels can realistically be produced in the United States, and how the development of these fuels will affect the rural sector.

References

- Aldrich Catalog Handbook of Fine Chemicals*, Milwaukee WI, 1994.
- Ali, Y., Hanna, M.A. and S.L. Cuppert. "Fuel Properties of Tallow and Soybean Oil Esters," *Journal of Am. Oil Chem. Soc.*, 72(1995):1557-1564.
- American Biofuels Association. *Biodiesel: A Technology, Performance, and Regulatory Overview*, Information Resources, Inc., February 1994.
- American Petroleum Institute. *API Technical Data Book, Petroleum Refining*, Washington, DC, August 1964.
- American Society for Testing Materials (ASTM). West Conshohocken, PA. Various issues.
- Ash, M., Douvelis, G., Castaneda, J., and N. Morgan. *Oilseeds: Background for 1995 Farm Legislation*. AER No. 715. Economic Research Service, U.S. Dept. Agr., May 1995.
- Biofuels Update*. "Report on U.S. Department of Energy Biofuels Technology," Volume 5, Issue 1, Winter 1997.
- _____. Volume 4, Issue 3, Fall 1996.
- Burton, J.W., Israel, D.W., Wilson, R.F., and T.E. Carter, Jr. "Effects of Defoliation on Seed Protein Concentration in Normal and High-Protein Lines of Soybean." *Plant and Soil* 172(1995):131-139.
- Burton, J.W. "Development of High-Yielding High-Protein Soybean Germplasm." p. 109-117. In: Wilson, R.F. (ed.). *Designing Value-Added Soybeans for Markets of the Future*, Am. Oil Chem. Soc., Champaign, IL, 1991.
- Cambridge Energy Research Associates. *1995 World Oil Trends*. Arthur Anderson & Co., S.C. 1995 Edition.
- Carlson, K.D., and D.L. Van Dyne. *Industrial Uses For High Erucic Acid Oils From Crambe and Rapeseed*. Extension and Agricultural Information Office, University of Missouri, Columbia. October 1992.
- Chapman, S.R., and L.P. Carter. *Crop Production*. W.H. Freeman and Company, San Francisco, 1976.
- Clark, S., Wagner, L., Schrock, M., and P. Piennaar. "Methyl and Ethyl Soybean Esters as Renewable Fuel for Diesel Engines," *Journal of American Oil Chemist's Society*, 10(1984):1632.
- Cruz, O., Stanfill, J., and B. Powaukee. "Pilot Production of Biodiesel on the Nez Perce Tribe Reservation," *Proceedings of the Seventh National Bioenergy Conference, The Southeastern Regional Biomass Energy Program*, September 15-20, Nashville, Tennessee, Vol 1, p. 364-371.
- Dehesh, K., Edwards, P., Hayes, T., Cranmer, A., and J. Fillatti. "Two Novel Thioesterases are Key Determinants of the Bimodal Distribution of Acyl Chain Length of *Cuphea Palustris* Seed Oil," *Plant Physiol*, 110(1996):202-210.
- Dewey, R.E., Wilson, R.F., Novitzky, W.P., and J.H. Good. "The AAPT1 Gene of Soybean Complements a Cholinephosphotransferase-Deficient Mutant of Yeast." *Plant Cell* 6:(1994) 1495-1507.
- Dunn, R.O., Shockley, M.W., and Bagby, M.O. "Improving the Low-Temperature Properties of Alternative Diesel Fuels: Vegetable Oil-Derived Methyl Esters." *Journal of Amer. Oil Chem. Soc.*, 73(1996):1719-1728.
- Engine Manufacturers Association (EMA). *Biodiesel Fuels and Their Use in Diesel Engine Applications*. Authored by the Alternative Fuels Committee of the Engine Manufacturers Association. Engine Manufacturers Association, Chicago, IL, August 1995.
- Federal Register*. Vol. 61, No 205, October 22, 1996, p. 54797. Government Printing Office, Washington D.C.
- _____. Vol. 61, No 51, March 14, 1996, p. 10623. Government Printing Office, Washington D.C.
- _____. Vol. 62, No 135, July 15, 1997, p. 37898. Government Printing Office, Washington D.C.
- Feedstuffs: The Weekly Newspaper for Agribusiness*, The Miller Publishing Company, Minnetonka, MN. 1992-94.
- Foglia, T.A., Nelson, L.A., Dunn, R.O. and W. N. Marmer. "Low-Temperature Properties of Alkyl Esters of Tallow and Grease," *Journal of Amer. Oil Chem. Soc.*, 74(1997):951-955.
- Graboski, M.S., and R.L. McCormick. "Combustion of Fat and Vegetable Oil Derived Fuels in Diesel Engines,"

Progress in Energy and Combustion Science, 24(1998):125-164.

Graboski, M.S., McCormick, R.L., and J.D. Ross. "Transient Emissions From No. 2 Diesel and Biodiesel Blends in a DDC Series 60 Engine," *Society of Automobile Engine Technology*, Technical Paper No. 961166, 1996.

Haines, H. "Truck in the Park Demonstration," Conference Proceedings, *Commercialization of Biodiesel: Establishment of Engine Warranties*, November 9-10, 1994, p.77-87. Edited by C.L. Peterson, National Center for Advanced Transportation Technology, University of Idaho, Moscow, Idaho.

Harrington, K. "Chemical and Physical Properties of Vegetable Oil Esters and Their Effect on Diesel Fuel Performance," *Biomass*, 9(1986):1-17.

Heywood, J. *Internal Combustion Engine Fundamentals*, McGraw-Hill, New York, 1988.

Howell, S. *Lubricity of Biodiesel Fuel: Update No. 3*. Report to National Biodiesel Board, Kansas City, MO, July 1994.

Israel, D.W., and J.W. Burton. "Nitrogen Nutrition of Soybean Grown on Coastal Plain Soils of North Carolina." North Carolina Agricultural Research Service. Technical Bulletin 310. 1997.

Krisnangkura, K. "Estimation of Heat of Combustion of Triglycerides and Fatty Acid Methyl Esters," *Journal of the American Oil Chemist's Society*, 68(1991):56-58.

Kinney, A.J. 1997. "Development of Genetically Engineered Oilseeds." pp 298-300. In: *Physiology, Biochemistry and Molecular Biology of Plant Lipids*, Williams, J.P., Khan, M.U., and N.W. Lem, eds., Kluwer Academic Publishers, Dordrecht, Holland.

Lee, H., and R. Conway. "The Ethanol Market: Facing Challenges and Opportunities," *New Crops, New Uses, New Markets, 1992 Yearbook of Agriculture*, pp 221-230. U.S. Dept. Agriculture.

Lee, I., Johnson, L.A., and E.G. Hammond. "Reducing the crystallization temperature of biodiesel by winterizing methyl soyate." *Journal of the American Oil Chemist's Society*, 73(1996)631-636.

Midwest Biofuels Inc. *Biodiesel Pour Point and Cold Flow Study*. Report to National Soydiesel Development Board, September 30, 1993, St. Louis, MO.

Milling & Baking News. "The Newsweekly of Grain-Based Foods." 1992-94. Sosland Publishing Co., Kansas City, MO.

Moon, H., Scowby, M., Avdiushko, S., and D. Hildebrand. "Effect of a Mammalian Desaturase on Specific Lipids in Transgenic Plant Tissues." p. 377-379. In Williams, J., Khan, M., and N. Lem (eds.). *Physiology, Biochemistry and Molecular Biology of Plant Lipids*. Kluwer Academic Publishers, Dordrecht, Holland, 1997.

Morgan, N. "World Vegetable Oil Consumption Expands and Diversifies," *Food Review*, Vol. 16, Issue 2, May-August 1993. Economic Research Service, U.S. Dept. Agriculture.

Nelson, L.A., Foglia, T.A., and W. Marmer. "Lipase-Catalyzed Production of Biodiesel," *Journal of Amer. Oil Chem. Soc.*, 73(1996):1191-1195.

New Fuels & Vehicles Report. Volume 19, No. 8, April 10, 1998, Washington, D.C.

Pantalone, V.R., Burton, J.W., and T.E. Carter, Jr. "Soybean fibrous root heritability and genotypic correlations with agronomic and seed quality traits." *Crop Science*. 36(1996a):1120-1125.

Pantalone, V.R., Burton, J.W., and T.E. Carter, Jr. "Soybean Seedling Grafting Technique." *Soybean Genetics Newsletter*. 23(1996b):203-205.

Pantalone, V.R., Rebetzke, G.J., Burton, J.W., and R.F. Wilson. "Genetic Regulation of Linolenic Acid Concentration in Wild Soybean Glycine Soja Accessions." *Journal of the American Oil Chemist's Society*, 74(1997):159-163.

Peterson, C.L., Auld, D.L., and R.A. Korus. "Winter Rape Oil Fuel for Diesel Engines: Recovery and Utilization," *Journal of the American Oil Chemist's Society*, 60(1983):1579-1587.

Peterson, C.L., Reece, D.L., Hammond, B.J., Thompson, J., and S.M. Beck. "Processing, Characterization and Performance of Eight Fuels From Lipids," ASAE Paper No. 946531, An ASAE Presentation, 1994 ASAE

International Winter Meeting, Atlanta, Georgia, December 13-16, 1994.

Peterson, C., Reece, D., Thompson, J., and Z. Zhang. *Development of Rapeseed Biodiesel for Use in High-Speed Diesel Engines*. University of Idaho, Department of Biological and Agricultural Engineering, Idaho Agricultural Experiment Station, University of Idaho, No. 302, September, 1996. Progress report for the U.S. Department of Energy, Bonneville Power Administration, Contract Number 93BIO9233.

Ryan, T., Dodge, L. and T. Callahan. "The Effects of Vegetable Oil Properties on Injection and Combustion in Two Different Diesel Engines," *Journal of the American Oil Chemist's Society*, 61(1984):1610.

Schafer, A. "Environmental and Health Concerns at Mercedes Benz," Conference Proceedings, *Commercialization of Biodiesel: Environmental and Health Benefits*, May 21-22, 1996, p.199-209. Edited by Charles L. Peterson, National Center for Advanced Transportation Technology, University of Idaho, Moscow, Idaho, 1997.

Sanford, S. and Evans, S. *Peanuts: Background For 1995 Farm Legislation*. AER No. 710. Economic Research Service, U.S. Dept. Agr., April 1995.

Sharp, C. *Transient Emissions Testing of Biodiesel in a 6V-92TA DDEC Engine*. Southwest Research Institute Report No. 6602 and 6673 to National Biodiesel Board, October 1994.

Sharp, C. *Transient Emissions Testing of Biodiesel and Other Additives in a DDC Series 60 Engine*. Southwest Research Institute Report for the National Biodiesel Board, December 1994.

Sharp, C. *Emissions and Lubricity Evaluation of Rapeseed Derived Biodiesel Fuels*. Southwest Research Institute Report for Montana Department of Environmental Quality, Helena, Montana, November 1996.

Sheehan, J., Camobreco, V., Duffield, J., Graboski, M., and H. Shapouri. *Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus*. National Renewable Energy Laboratory, NREL/SR-580-24089, UC Category 1503, Golden, Colorado, May 1998.

Swenson, A.L., Johnson, R.G., Helgeson, D.L., and K.R. Kaufman. *Economics of Producing Sunflower For*

Fuel On Diverted Acres, Department of Agricultural Economics, North Dakota State University, Agricultural Economics Report No. 176, September 1983.

University of Idaho. *Conference Proceedings, Commercialization of Biodiesel: Environmental and Health Benefits*, Mammoth Hot Springs, Yellowstone National Park, May 21-22, 1996. Edited by Charles L. Peterson, Department of Biological and Agricultural Engineering, University of Idaho, 1997.

U.S. Department of Agriculture, Agricultural Research Service. *Vegetable Oil As Diesel Fuel, Seminar III*, Agricultural Reviews and Manuals ARM-NC-28, October 19-20, 1983.

U.S. Department of Agriculture, Economic Research Service. *Oil Crops Yearbook*. October, 1996 and 1997.

_____. *Industrial Uses Of Agricultural Materials Situation and Outlook Report*. IUS-6, August 1996, p 22-23.

_____. *Agricultural Outlook*. AO-242, July 1997.

U.S. Department of Agriculture, National Agricultural Statistics Service (USDA/NASS). *Crop Production: 1995 Summary*. Cr Pr 2-1(96), Jan. 1996.

_____. *Crop Production: 1994 Summary*. Cr Pr 2-1(95), Jan. 1995.

_____. *Crop Production: 1993 Summary*. Cr Pr 2-1(94), Jan. 1994.

_____. *Livestock Slaughter Summary*. Mt An 1-2-1(95), March 1995.

U.S. Department of Energy, Energy Information Administration (EIA). *Monthly Energy Review*. DOE/EIA-0035, April 1997.

U.S. Department of Commerce, Bureau of the Census. *1992 Census of Agriculture*. Geographic Area Series, Vol. 1, Nov. 1994.

_____. *Fats and Oils-Production, Consumption, and Stocks*. Annual Summaries 1993-95.

U.S. Department of Transportation, Federal Highway Administration. *Monthly Motor Fuel Reported by States, September 1997*. Publication No. FHWA-PL-98-001.

Van Gerpen, J. "Cetane Number Testing of Biodiesel," *Liquid Fuels and Industrial Products From Renewable Resources*. Proceedings of the Third Liquid Fuel Conference, September 15-17, 1996, Nashville, Tennessee.

Van Gerpen, J. Unpublished Research at Iowa State University, 1994, Ames, Iowa.

Voelker, T., Worrell, A., Anderson, L., Bleibaum J., Fan, C., Hawkins, D., Radke, S., and H. Davies. "Fatty Acid Biosynthesis Redirected To Medium-Chain in Transgenic Oilseed Plants," *Science*, 257(1992):72-74.

Walton, J., *Gas & Oil Power*, Whitehall Press, London, England, July(1938):167.

Wilson, R.F., Burton, J.W., Kwanyuen, P., and D.W. Israel. "Developing High-Protein High-Oil Soybean Varieties." Proc. UJNR Protein Panel. 24:25-30. 1995.

Withers, R.V., and M. Noordam. "Biodiesel From Canola: An Economic Feasibility Analysis," *Liquid Fuels and Industrial Products From Renewable Resources*. Proceedings of the Third Liquid Fuel Conference, September 15-17, 1996, Nashville, Tennessee.

Appendix

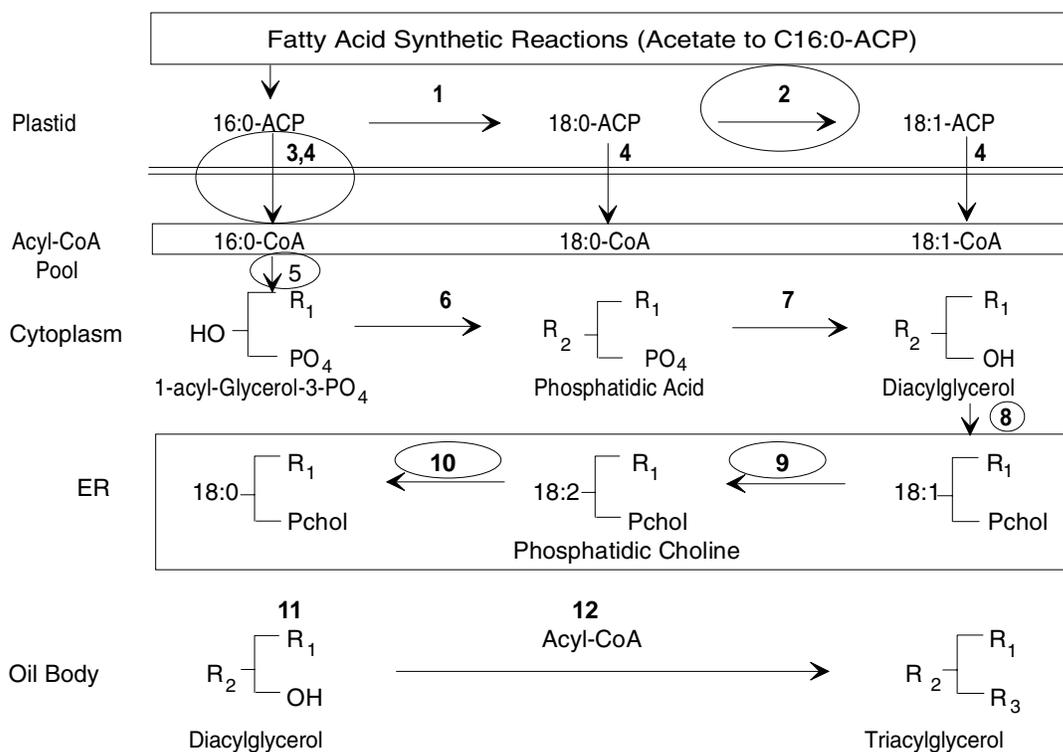
Developing genetic modification strategies for fatty acids in the production of triacylglycerol requires an understanding of how a plant makes oil, i.e., triacylglycerol biosynthesis (fig. A). The pathway to seed oil production involves 12 enzyme reaction steps that an oil plant follows to form triacylglycerol. Enzymes catalyze specific biochemical reactions at each of the 12 steps (table A). This process in an oilseed begins with fatty acid synthesis in plastids, which produces the 16-carbon precursor of palmitic acid, 16:0-acyl-carrier-protein (ACP). The reaction that converts palmitic acid to stearic acid (18:0-ACP) is found at step 1. At step 2, stearic acid (18:0-ACP) is converted to oleic acid (18:1-ACP). Two thioesterases at steps 3 and 4 then convert 16:0 acyl-ACP to acyl-co-enzyme-A molecules that are used to make the phospholipids, lyso-phosphatidic acid (step 5) and phosphatidic acid (step 6). At step 7, a phosphatase converts phosphatidic acid (PA) to diacylglycerol (DG), which is converted to phosphatidyl choline (Pchol) in the endoplasmic

reticulum of plant cells (step 8). Although other phospholipids also are formed from DG, PC is the primary substrate for -6 and -3 desaturases that catalyze synthesis of the polyunsaturated linoleic (18:2) and linolenic (18:3) acids (steps 9 and 10). PC is then converted back to DG at step 11, and another acyl-co-enzyme-A is added to DG to form triacylglycerol (step 12) in the final reaction of this pathway.

Scientists use the triacylglycerol biosynthesis pathway as a guide to help them develop new varieties of oil plants. Through plant breeding and genetic engineering, desirable enzyme reactions can be increased while undesirable reactions are diminished. For example, the reaction rate at step 9 that converts oleic to linoleic acid can be reduced to increase the cetane number of an esterified oil used for biodiesel production. Cetane number can also be increased by increasing the conversion rate of linoleic to linolenic acid at step 10 in the glycerolipid synthetic pathway (fig. A). Enzyme reactions at other steps that may enhance the quality of biodiesel are circled (fig. A).

Figure A

Simplified diagram of triacylglycerol biosynthesis in plants



Where:

- Acetate is a salt of two-carbon carboxylic acid that is the metabolic building block of fatty acid molecules;
- Plastids are organelles related to chloroplasts in cells of leaves;
- Acyl is a term for a hydrocarbon chain with a fatty acid;
- Cytoplasm, a protein matrix or gel, contains cell parts and enzymes essential to cellular life;
- HO at step 5 shows a hydroxyl group attached to a carbon atom in a lipid molecule;
- OH at step 7 shows a hydroxyl group attached to a carbon atom in a lipid molecule;
- PO₄ shows a phosphate group attached to a phospholipid molecule;
- Phosphatidic Acid is a phosphorylated molecule of glycerol carrying two fatty acids, which is the central

metabolic intermediate for both phospholipid and triacylglycerol synthesis;

- Lyso-phosphatidic acid is a phosphorylated molecule of glycerol carrying one fatty acid that is the direct metabolic precursor of phosphatidic acid;
- ER (endoplasmic reticulum) is the organelle within plant cells where oil is made;
- Phcol (phosphatidyl choline), a major phospholipid, is a molecule containing fats and phosphorus compounds;
- Oil body is a membrane-bound cellular inclusion that contains triacylglycerol;
- R₁ is a fatty acid attached to the stereospecific position 1 of a glycerolipid;
- R₂ is a fatty acid attached to the stereospecific position 2 of a glycerolipid; and
- R₃ is a fatty acid attached to the stereospecific position 3 of a glycerolipid.

Table A—Twelve enzyme steps of triacylglycerol biosynthesis in plants

Step	Enzyme	Function of reaction
1	3-keto-acyl-ACP synthetase II	Catalyzes stearic acid synthesis
2	18:0-ACP desaturase	Catalyzes oleic acid synthesis
3	Acyl-ACP thioesterase	Converts 16:0 or 18:0-ACP to acyl-CoA
4	Acyl-ACP thioesterase	Converts 18:1-ACP to 18:1-CoA
5	Glycerol-3-PO ₄	Catalyzes lyso-phosphatidic acid synthesis
6	1-acylglycerol-3-PO ₄	Catalyzes phosphatidic acid synthesis
7	L- α -phosphatidate phosphatase	Catalyzes diacylglycerol (DG) synthesis
8	CDP-choline:sn-1,2 diacylglycerol cholinephosphotransferase	Catalyzes phosphatidylcholine synthesis
9	ω -fatty acid desaturase ¹	Catalyzes linoleic acid synthesis
10	ω -fatty acid desaturase ¹	Catalyzes linolenic acid synthesis
11	Phospholipase ² C	Converts phosphatidylcholine to DG
12	Diacylglycerol acyltransferase	Catalyzes triacylglycerol or oil synthesis

¹ ω indicates location of unsaturated bond position from the methyl carbon end of the fatty acid.

²This enzyme cleaves a phospholipid to give a molecule of diacylglycerol plus the phosphate polar group.